1.0

1.1

1.25 | 1.4 | 1.6

2.8 | 2.5
3.2 | 2.2
3.6
4.0 | 2.0

1.8

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963-A

AD-A183 698

FIELD APPLICABLE METHOD TO REDUCE DENTAL EMERGENCIES

FINAL REPORT

NORMAN TINANOFF, D.D.S., M.S.
DAVID CAMOSCI, B.S.
MARY MANWELL, B.S.

JULY 31, 1987

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland   21701-5012

DTIC
ELECTE
AUG 2 5 1987
E

University of Connecticut Health Center
Farmington, Connecticut   06032

87   8 20   039

BLOCK 20

In vitro experiments have shown that twice daily exposure of S. mutans to various fluoride salts has shown that only SnF_2 significantly alters this organisms growth and metabolism. The antibacterial effect of SnF_2 was associated with an uptake of tin into the bacteria. Fluoride salts of sodium, lead, zinc, and copper had little effect in this test system. The pH of the various fluoride salts generally had no effect on the test compound activity except for the noted inactivation of SnF_2 at elevated pH's. Since SnF_4 also did not affect the growth or metabolism of S. mutans, a unique property of SnF_2, possibly its reactivity in an aqueous environment, may be responsible for its antibacterial properties.

Stannous fluoride was compared to NaF (5 ppm F^-) in the drinking water of hamsters to test whether SnF_2 had greater caries inhibitory effects due to its potential antibacterial effects. The number of enamel and dentinal carious lesions in both the NaF and SnF_2 group was significantly different from the deionized water group; however, there was no difference in caries scores between the NaF and SnF_2 group. The recovery of S. mutans was highly variable between animals and showed no statistical difference between groups.

Twenty-two human subjects, who were regarded as potentially caries active, rinsed twice a day with either acidulated NaF or SnF_2 mouthrinses, adjusted to 200 ppm F^-. There was a small (2 times) but significant reduction in Total CFU per ml saliva in both groups after a year. No differences were found in lactobacilli counts between the 2 mouthrinse groups or longitudinally within the groups. Of importance the apparent selective reduction in S. mutans found in those subjects rinsing with SnF_2. At the end of 1 year, the SnF_2 group had less (26 times) fewer S. mutans compared to baseline. With regard to caries, all patients continued to be caries active after one year despite the use of two daily fluoride mouthrinses; however, the subjects rinsing with SnF_2 developed approximately half the number of new carious lesions to those subjects rinsing with acidulated NaF. With regard to gingival health, this study did find that SnF_2 was an adjunct in decreasing gingival inflammation. The lower frequency of bleeding sites and the corresponding lower mean GI scores in the SnF_2 group compared to the NaF group demonstrates that rinsing with SnF_2 favorably affected gingival health.

The controlled release clinical trials were designed to examine the safety and efficacy of a controlled release delivery system of SnF_2 in a small number of human subjects. The SnF_2 restorations showed no signs of wear or loss of integrity in both the trials. The salivary fluoride release rate was found to average 0.3 ppm over the 34 day trial. Some effect on both the quantity and proportion of microorganisms was noted in those subjects who had a SnF_2-polycarboxylate restorations in place. While there was an increase of recovery of total colony forming units from salivary in the placebo group during the experimental period, probably due to suspension of oral hygiene in this period, a decrease in total bacteria was noted in the SnF_2 group. This decrease in salivary microorganisms may be selective since S. sanguis recoveries showed no difference between groups while S. mutans recoveries appeared less in those subjects having the SnF_2 restoration. The effect of the SnF_2 delivery system against plaque and gingivitis was not impressive.

A183 698

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION	1b. RESTRICTIVE MARKINGS
UNCLASSIFIED	

2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT
	Approved for public release;
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	distribution unlimited.

4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION	6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
University of Connecticut Health Center		

6c. ADDRESS (City, State, and ZIP Code)	7b. ADDRESS (City, State, and ZIP Code)
Farmington, Connecticut 06032	

8a. NAME OF FUNDING/SPONSORING ORGANIZATION	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
U.S. Army Medical Research & Development Command		DAMD17-81-C-1075*
		DAMD17-78-C-8066**

8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS			
Fort Detrick	PROGRAM ELEMENT NO.	PROJECT NO. 3S1-	TASK NO.	WORK UNIT ACCESSION NO.
Frederick, Maryland 21701-5012	62775A	62775A825	AC	080

11. TITLE (Include Security Classification)

(U) Field Applicable Method to Reduce Dental Emergencies

12. PERSONAL AUTHOR(S)
Norman Tinanoff, David Camosci, Mary Manwell

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 9/1/78 TO 9/30/83	1987 July 31	

16. SUPPLEMENTARY NOTATION
*Period of time 1 August 1981 - 30 September 1983
**Period of time 1 September 1978 - 30 June 1981

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Dental emergencies; Field applications; Antibacterial;
06	05		Delivery system; Fluoride
06	13		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

See next page.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Mary Frances Bostian	301-663-7325	SGRD-RMI-S

DD Form 1473, JUN 86   *Previous editions are obsolete.*

UNCLASSIFIED

Table of Contents

Accession For

NTIS GRA&I   X
DTIC TAB   ☐
Unannounced   ☐
Justification

By
Distribution/

Availability Codes

Dist | Avail and/or Special

DTIC COPY INSPECTED 6

A-1

Table of Contents (continued)

Summary

A series of experiments have been performed to try to find the best
antimicrobial fluoride compound. We have extensively examined 8 cationic
salts of fluoride, some with higher or lower molecular weights than tin.
Because it quite early became apparent that pH of the compounds was impor-
tant, over 26 variations of pH of these compounds were tested. To date, we
have found that SnF_2 has potent antimicrobial activity, but remarkably, $SnCl_2$
and SnF_4 does not. ZnF_2, $ZnCl_2$, PbF_2, $PbCl_2$ and NaF all have no real effect
on microbiology. Concerning pH, SnF_2 appears to have a critical pH below 4
for its antimicrobiologic effect. The loss of effectiveness above pH 4 we
feel is due to its conversion to insoluble tin hydroxide salts which cannot
affect bacteria. Atomic absorption spectrophotometric and electron micro-
probe experiments have shown us that only SnF_2 accumulates within bacterial
cells which causes the bacteria to have unbalanced growth characteristics
and hence the antibacterial effect.

We have completed all the planned in vitro and pilot studies on the
controlled release SnF_2 system. These studies have involved: (1) the in
vitro microbiologic effects of low levels of SnF_2; (2) in vitro physical
properties of SnF_2-polycarboxylate cement; (3) an in vivo trial for 30 days
in one subject examining the antiplaque effects, release levels, systemic
effects, and the clinical integrity of the restoration. These studies have
all been favorable in moving forward in developing the controlled release
system. The studies have been written, submitted for publication.

Two pilot studies on hamsters have been completed to date. One large
experiment involving 45 animals is presently in progress. In the first
pilot study, 4 hamsters had their molars prepared to accept the SnF_2 poly-
carboxylate cement or polycarboxylate cement without additional fluoride.
After the restorations were placed, the hamsters were innoculated with S.

1

mutans and placed on a cariogenic diet for 60 days. Those hamsters which had the SnF_2 temporary showed a trend for fewer carious lesions and fewer S. mutans; however, the temporary restorations were completely absent from all preparations upon sacrifice. Another pilot study was performed to try to improve on the technique of placing the restorations into the preparation. Besides not being able to adequately fill the preparation because of the small size and air bubbles, we found that many of the animals would die due to the extended time their jaws were open. Since the objective in the animal experiment was to see the microbiologic effects of low levels of SnF_2, and not manipulative techniques of the cement in tiny restorations, it was decided to first perform an experiment with controlling the exposures of SnF_2 through the animals drinking water. This experiment, with 15 animals each in a deionized water group, a 5 ppm F^- groups as NaF, and a 5 ppm F as SnF_2 group are currently underway.

We have applied to the Food and Drug Administration for an "Investigational Exemption for a New Drug" (FDA 571) so that we can utilize the controlled release delivery system of SnF_2 to human clinical trials. The application and research protocol was submitted February 4, 1982. So far it has not, to my knowledge, been approved. These studies are projected to start November 1, 1982. The human experiments (not included in original contract) involve a short term (2 week) study to examine efficacy and a long term trial (2 months).

As a pilot study, we have selected 2 subjects with moderate generalized periodontititus. On one side of the mouth, we have injected 1% SnF_2 subgingivally with a 25 gauge needle. To the other side, we have injected saline. On a weekly basis, we have sampled the pockets for periodontopathic anaerobes and S. mutans. We have found that in both subjects, the pockets that were irrigated with SnF_2 had much fewer B. melinenogenicus and S. mutans,

2

even 4 weeks after the single treatment. A larger clinical study to follow-up these preliminary results is planned.

After initially starting with 36 rampant caries subjects, only 18 remain compliant with using either SnF_2 or NaF twice a day in our long term mouthrinse study. After 6 months, many significant differences between the groups have been noted. With regard to clinical parameters, the group rinsing with SnF_2 had significantly less plaque at one month and the gingival health improved significantly more between 1 and 3 months with these subjects rinsing with SnF_2. The most dramatic finding is the low variance in gingival scores in those subjects rinsing with SnF_2. With regard to caries scores, we have found that the group rinsing with NaF had 4.2 new lesions after 1 year and those subjects rinsing with SnF_2 had 2.3 new lesions. Microbiologically, there are large differences between groups. Those subjects rinsing with SnF_2 had 2.4 times fewer bacteria/ml saliva in their mouths at 3 months. But, of most importance, was the fact that the SnF_2 users had greater than 20 times fewer S. mutans in their mouth over the course of the study.

Table 1: Enamel cylinders (n = 4/treatment group) inoculated with S. mutans NCTC 10449 and exposed to various fluorides and controls for 1 min. twice a day for 2 days. Plate counts used to determine the number of bacteria attached to enamel after 2 days, turbidity measurements used to determine amount of bacterial material attached to enamel after 2 days.

Treatment	PLATE COUNTS			SPECTROPHOTOMETRY			Correlation of plate counts and spec. readings
	Counts (10^7) $\bar{x} \pm$ S.D.	% dif. from H_2O	Subset α group	Spec. readings $\bar{x} \pm$ S.D.	% dif. from H_2O	Subset α group	
H_2O (0.0 ppmF)	95.6±31.0	--	1	.366±.107	--	1	0.99
NaF (100 ppmF)	81.6±12.9	-11.5	1	.358±.058	- 2.2	1	0.79
$SnCl_2$ (100 ppm Sn)	129.5±71.5	+36.3	1	.450±.135	+22.8	1	0.56
Na_2SnF_6 (100 ppmF)	134.8±48.3	+41.0	1	.434±.081	+18.6	1	0.24
SnF_2 (100 ppmF)	26.8±14.4	-71.9	2	.172±.087	-53.1	2	0.97
SnF_2 (250 ppmF)	1.4± 0.8	-98.5	3	.046±.009	-87.3	3	0.88

α Homogenous subsets using Analysis of Variance with Duncan procedure ($p \leq 0.05$).

4

Sterile Stainless Steel Wires

Suspended in Jordan's Medium

+

Inoculated with S. mutans

48 hours

Exposed 1 min.

HOH SnF_2 $SnCl_2$
           (250 ppm)   (Sn ppm = SnF_2)

Rinsed 1 min.

Fresh Jordan's Medium

12 hours ----→ pH

Repeat Exposure

12 hours ----→ pH

Repeat Exposure

12 hours

pH (terminal)

Plaque Score

5-day Preformed Plaque

Repeat Exposure (2 min.)   Collect Plaque

24 hrs. ----→ pH

Repeat Exposure (2 min.)   Dry Plaque ——→ mg

24 hrs. ----→ pH

Repeat Exposure (2 min.)   Atomic Absorption ——→ Sn/mg

24 hrs   pH (terminal)

Plaque Score

Figure 2: Flow diagram used to test the effect of SnF_2 and $SnCl_2$ on 2
            and 5 day old preformed S. mutans plaques. Deionized H_2O was
            the control.

5

Table 2: List of the solutions at various pH's which were exposed (1 min./12 hrs. for 48 hrs.) to wire adherent $\underline{S.}$ $\underline{mutans}$ NCTC 10449. Fluoride solutions were tested at 250 parts/ 10^6 F$^-$, except for PbF$_2$ which was tested at 100 part/10^6 F$^-$. The cations in the comparable chloride salts were equal in ppm to the fluoride salts.

Agent	Cation (ppm)	Anion (ppm)	pH
NaF	303	250	2.0, 2.5, 3.0, 5.5, 6.0, 7.0.
SnCl$_2$	783	463	2.5, 7.0.
SnF$_2$	783	250	2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0.
SnF$_4$	390	250	2.3, 5.0.
ZnF$_2$	428	250	5.2
ZnCl$_2$	428	465	4.9
PbF$_2$	565	100	3.0, 6.0
PbCl$_2$	545	187	3.0, 6.0
H$_2$O	---	---	2.5, 7.0

Table 3: Initial study of intermittent exposures (1 min./12 hrs. for 48 hrs.) of tin and/or fluoride solutions on acid production, plaque formation, and tin accumulation of wire adherent S. mutans NCTC 10449. Deionized water (pH 2.5 and 7.0) was used as a control. Fluoride solutions at 250 ppm F^-; cations in $SnCl_2$ equal to SnF_2.

	Agent pH	Acid Production $(\Delta\ pH)$	Plaque Score[a]	Plaque Weight (mg)	Sn/mg Plaque (µg)
H_2O	2.5	2.8	4	11.0 ± 0.2	N.D.[b]
	7.0	2.7	4	10.7 ± 0.5	N.D.
NaF	2.5	2.5	4	12.5 ± 0.5	N.D.
	7.0	2.7	4	11.2 ± 0.9	N.D.
$SnCl_2$	2.5	2.7	4	12.3 ± 0.6	1.4 ± 0.6
	7.0	2.7	4	11.1 ± 0.7	0.3 ± 0.1
SnF_2	2.5	1.7	2	7.2 ± 2.0	13.6 ± 4.1
	7.0	2.6	4	13.0 ± 0.5	0.5 ± 0.1

a Scored by McCabe method

b None detected

N = 3; $\bar{x}$ ± S.D.

Table 4: Effect of intermittent exposures (1 min./12 hrs. for 48 hrs.) of NaF (pH 2.0 and 6.0) and SnF$_2$ (pH 2.0 to 6.0) on acid production, plaque formation, and tin accumulation of wire adherent S. mutans NCTC 10449. Fluoride solutions at 250 ppm F$^-$.

	Agent pH	Acid Production (Δ pH)	Plaque Score[a]	Plaque Weight (mg)	Sn/mg Plaque (μg)
NaF	2.0	1.8	4	6.4 $\pm$ 0.9	N.D.[b]
	6.0	2.4	4	6.5 $\pm$ 0.3	N.D.
SnF$_2$	2.0	0.2	<1	1.8 $\pm$ 0.1	λ
	3.0	0.2	<1	2.4 $\pm$ 0.5	42.9 $\pm$ 7.1
	4.0	0.5	1	2.6 $\pm$ 0.5	36.9 $\pm$ 3.6
	5.0	1.0	3	5.7 $\pm$ 0.4	20.1 $\pm$ 0.5
	6.0	1.6	4	5.9 $\pm$ 0.8	3.6 $\pm$ 0.7

a Scored by McCabe method

b None detected

λ Laboratory accident

N = 3; $\bar{x} \pm$ S.D.

Table 5: Effect of intermittent exposures of PbF_2 and $PbCl_2$ compared to NaF on acid production, plaque formation, and lead accumulation of wire adherent <u>S. mutans</u> NCTC 10449. Test solutions adjusted to either pH 3.0 or 6.0. Fluoride solutions at 100 ppm F^-; cations in $PbCl_2$ equal to PbF_2.

	Agent pH	Acid Production (Δ pH)	Plaque Score[a]	Plaque Weight (mg)	Pb/mg Plaque (μg)
NaF	3.0	1.9	3	5.7 ± 0.2	N.D.[b]
	6.0	2.3	3	4.6 ± 1.0	N.D.
$PbCl_2$	3.0	2.0	3	6.1 ± 0.3	2.2 ± 0.2
	6.0	2.3	3	5.5 ± 0.9	1.2 ± 0.2
PbF_2	3.0	1.9	3	6.7 ± 0.3	3.3 ± 0.5
	6.0	2.0	3	5.5 ± 0.1	2.4 ± 0.7

[a] Scored by McCabe method

[b] None detected

$N = 3$; $\bar{x}$ ± S.D.

Table 6: Effect of intermittent exposures (1 min./12 hr. for 48 hrs.) of NaF, SnF_2, SnF_4, ZnF_2 and $ZnCl_2$ on acid production, plaque formation, and metal accumulation of wire adherent S. mutans NCTC 10449. Natural pH for all test solutions, except SnF_4 which was adjusted to pH 5.0. Fluoride solutions at 250 ppm F^-; cations in $ZnCl_2$ equal to ZnF_2.

	Agent pH	Acid Production (Δ pH)	Plaque Score[a]	Plaque Weight (mg)	Sn/mg Plaque (μg)
NaF	5.5	2.7	3	9.8 ± 0.6	N.D.[b]
SnF_2	3.5	0.4	<1	1.3 ± 0.4	39.1 ± 1.4
SnF_4	2.3	2.6	3	10.9 ± 0.2	8.9 ± 2.0
SnF_4	5.0	2.6	3	10.4 ± 0.3	6.1 ± 0.3
ZnF_4	5.2	2.8	3	10.0 ± 0.5	0.05 ± 0.01
$ZnCl_2$	4.9	2.9	3	9.0 ± 0.2	0.12 ± 0.07

[a] Scored by McCabe Method

[b] None detected

$N = 3$; $\bar{x} \pm$ S.D.

Table 7: Performed plaque of S. mutans NCTC 10449 was grown on wires for 2 days and then exposed every 12 hrs. for 1 min. to various agents for the next 2 days. The intermittent exposures to the wires continued for 3 more days, once a day for 2 min. Each wire was scored for plaque, then collected, dried, weighed and analyzed for tin. Fluoride solutions at 250 ppm F; cations in $SnCl_2$ equal to SnF_2.

	Acid Production (Δ pH)	Plaque Score[α]	Plaque Weight (mg)	Sn/mg Plaque (μg)
H_2O	3.2	4	18.9 $\pm$ 0.2	N.D.
$SnCl_2$	3.2	4	22.6 $\pm$ 4.7	3.2 $\pm$ 0.8
SnF_2	2.4	4	15.7 $\pm$ 0.8	>8

α Scored by McCabe method
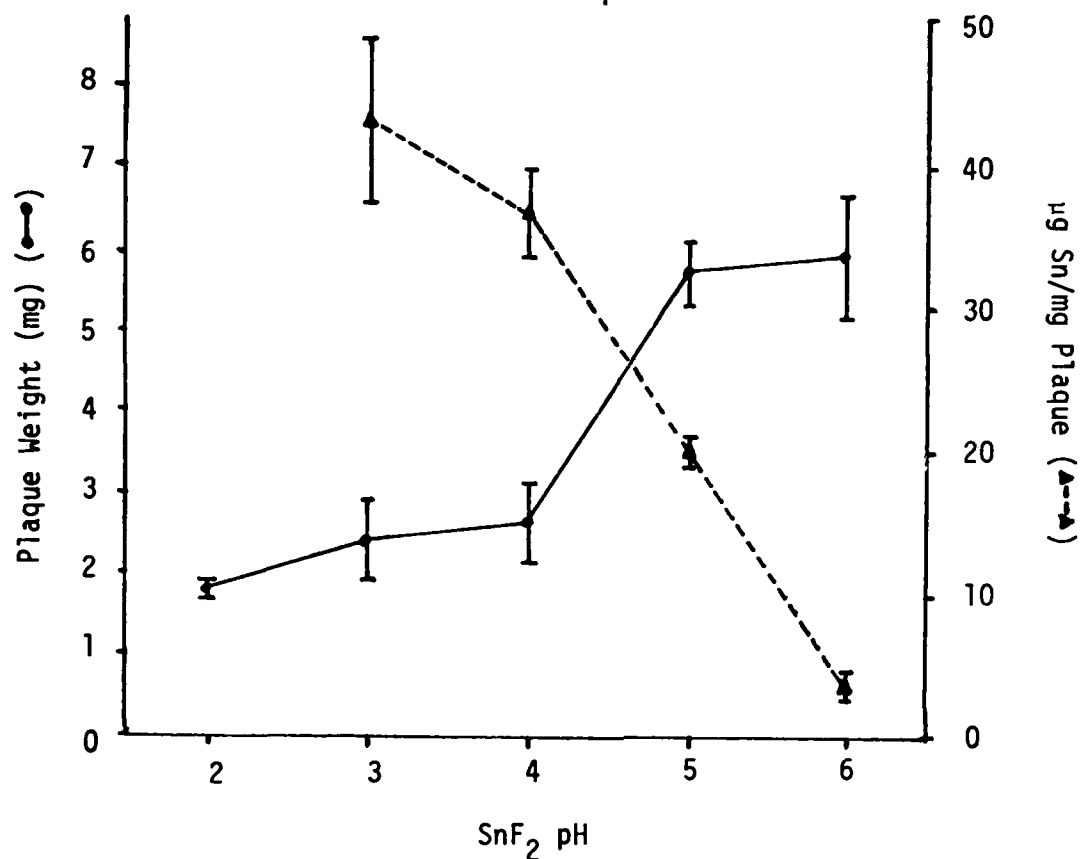
N.D. None detected

N=3; $\bar{x}$ $\pm$ S.D.

Figure 3: Intermittent exposure of SnF_2 (1 min./12 hrs. for 48 hrs.) at pH 2.0-6.0 on growth (plaque weight) and metal uptake (Sn/mg plaque). Reduction of bacterial growth by SnF_2 is inversely related to metal present in the bacteria at varying pH's.

Phase A: <u>Dental Materials Investigation and Testing</u>

> (See Appendix C - Antiplaque Properties of Sustained Release SnF_2:
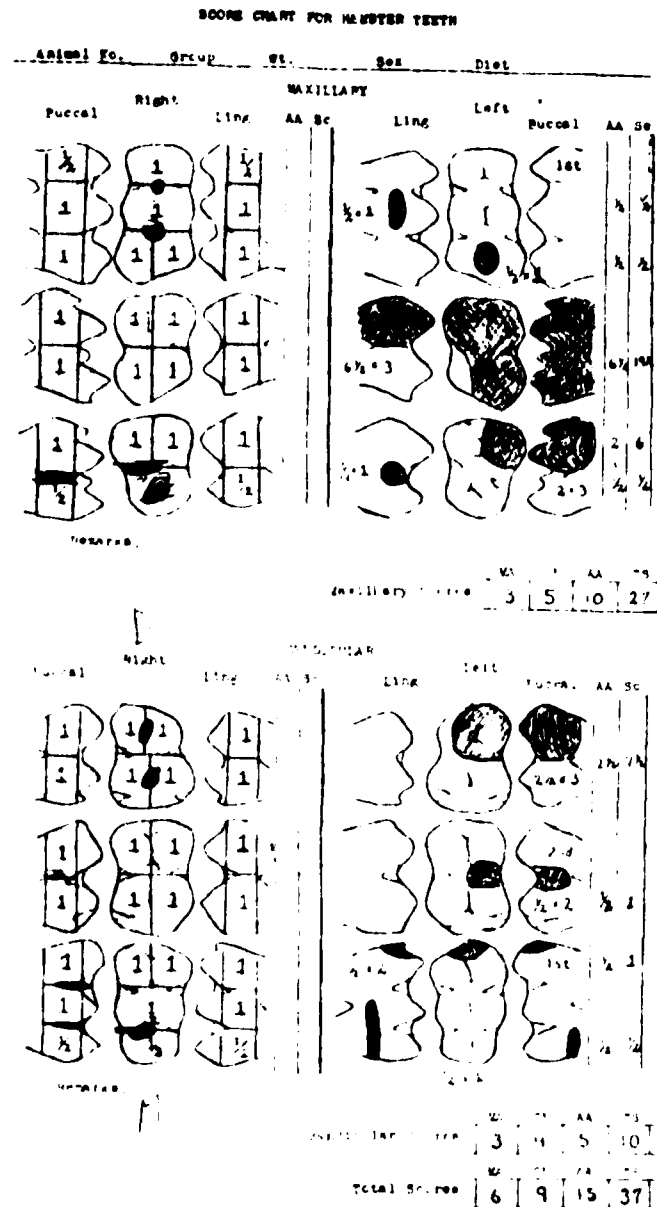>   Pilot Studies)

Phase B: <u>Measurement of Slow Release of Fluoride</u>

> (See Appendix C - Antiplaque Properties of Sustained Release SnF_2:
>   Pilot Studies)

Phase C: <u>Animal Studies</u>

Two pilot studies on hamsters have been completed. In the first pilot study, 4 hamsters had their molars prepared to accept the SnF_2 polycarboxylate cement or polycarboxylate cement without additional fluoride. After the restorations were placed, the hamsters were inoculated with <u>S. mutans</u> and placed on a cariogenic diet for 60 days. Those hamsters which had the SnF_2 temporary showed a trend for fewer carious lesions using the Keyes scoring techniques (Figure 4) and fewer <u>S. mutans</u> (Table 8); however, the temporary restorations were completely absent from all preparations upon sacrifice.

A second pilot study was performed to try to improve on the technique of placing the restorations into the preparation. Besides not being able to adequately fill the preparation because of the small size and air bubbles, we found that many of the animals would die due to the extended time their jaws were open. Since the objective in the animal experiment was to see the microbiologic effects of low levels of SnF_2, and not manipulative techniques of the cement in tiny restorations, it was decided to first perform an experiment with controlling the exposures of SnF_2 through the animals' drinking water. This experiment, with 15 animals each in a deionized water group, a 5 ppm F^- groups as NaF, and a 5 ppm F as SnF_2 group are currently underway.

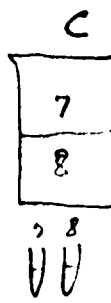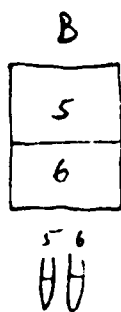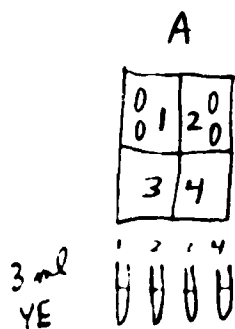Figure 4: Caries scoring charts for hamsters used in animal study, developed by Keyes, 1944.

14

Hamster Study Pilot Study
Estimate S. mutans.

12-15-81   24

A   B   C

3 ml
YE

10/23
0.2 ml 104495 S. mutans
Again, 1 wk later.

Sonicate Setting 4, 50 wts
Microtip 25 sec.
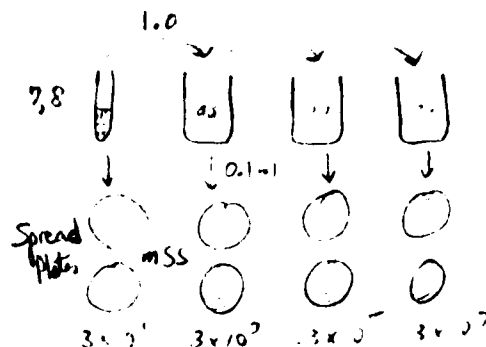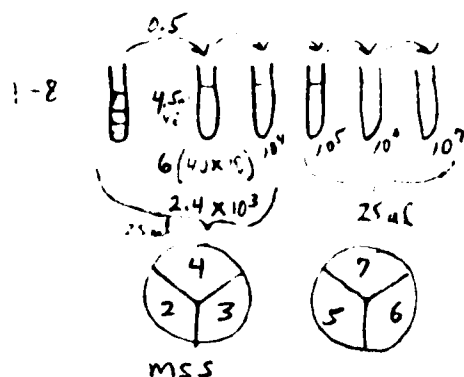
Supplies:
mss = 32 jars
glass = 2 tubes
plastic = 40 Tubes
YE = 800 ml

E   Raw Data   12-20-81

1:1 mss	L. Man	R. Man	Temp. R Max	Temp L Max	Man	Max	Man	Max	Pictures taken except #3
	1	2	3	4	5	6	7	8	
2	TN	TN	TN	TN	TN	TN	TN	TN	
3	TN	TN	TN	TN	TN	TN	TN	TN	
4	36,58	58,84	151,182	TN	TN	TN	TN	130,159	7:6
5	8,9	8,9	20,22	13,15	107,122	31,33	209,245	19,20	
6	0,1	0,1	0,1	4,5	14,16	4,5	19,20	1,2	
7	0,0	0,1	0,1	0,0	1,1	0,0	2,4	0,0	

Ave.
4	47	71	167	—	—	—	—	145	
5	9	9	21	14	117	32	227	20	
6	1	1	1	5	15	5	20	2	

Total
1.1 ×10^6	1.7 ×10^6	50 ×10^6	3.7 ×10^6	36 ×10^6	7.7 ×10^6	48 ×10^6	48 ×10^6	

Table 8: Results of first animal pilot study showing that hamsters which had slow released temporary restorations in place had fewer S. mutans; that is, mandibular teeth of experimental animals had approximately 1.5×10^6 S. mutans; whereas control animals had about 22×10^6 S. mutans on their mandibular molars.

Phase D: <u>Application for "New Investigational Drug Number"</u>

This aspect of the contract involves the administrative perusal of a "New Investigational Drug Number" from the Food and Drug Administration which will enable human experiments with controlled release SnF_2. This application has been submitted on February 4, 1982. On March 11, 1982, I received a letter explaining necessary revisions in the proposal. Subsequently, I re-submitted the proposal on March 25, 1982 (Appendix D).

Pilot Study to Examine the Effectiveness of Irrigating SnF_2
into Periodontally Diseased Pockets

As a pilot study, we have explored the possibility of reducing dental emergencies for soldiers in field situations by lavaging periodontal pockets with 1% SnF_2.

For this initial study, we identified 2 subjects which had generalized periodontitis and large numbers of Bacteroides melaninogenicus. After receiving consent (Figure 5), we irrigated the gingival pocket of one subject's tooth with 1% SnF_2 and on the contralateral side, we irrigated the gingival pocket with normal saline. One week later, in the pockets of both subjects which had been irrigated with SnF_2, no B. melaninogenicus could be detected. The sites that had been irrigated with saline, however, had 3.26×10^5 and 2.29×10^5 B. melaninogenicus, respectively, for each subject.

After 2 weeks, samples revealed that the experimental sites (SnF_2 treated) had 10 times fewer B. melaninogenicus. At 3 weeks, the differences were approximately 2 times.

This pilot study shows such favorable results that we plan to pursue these pilot results with further studies. Such a temporary treatment for acute periodontal problems could have important implications in a combat situation.

CONSENT FORM

School of Dental Medicine
University of Connecticut Health Center

Patient Consent Form for Fluoride Irrigation Study

Investigators: Norman Tinanoff, D.D.S., M.S.
                 Björn Klock, D.D.S., Dr. Odont.
                 Kenneth Kornman, D.D.S., Ph.D.

It has recently been shown that mouth rinses with fluoride solutions inhibit bacterial accumulation on teeth. Incorporated in a toothpaste the same type of fluoride (SnF_2) reduces gingivitis (gum inflammation). Periodontitis, i.e. loss of bone supporting the teeth, has also been shown to be less severe when the teeth were swabbed with an SnF_2 solution. The reason for these effects probably is that the microorganisms causing gingivitis and periodontitis are affected by SnF_2. We would like your permission to take part in a short-term clinical research experiment (no longer than 6 weeks) to measure the possible anti-bacterial effect of SnF_2 on specific microorganisms and on gingivitis when this agent is irrigated between the tooth and the gum tissues.

Procedure:

Bacterial accumulation, plaque, will be collected from two of your teeth twice a week over a six week period. We will also estimate the degree of gingival inflammation at each sampling occasion. At one of your first appointments the pockets around the two selected teeth will be irrigated with 2ml of 0.4% SnF_2 under minimal pressure. No further treatment, just scoring, will be done during the test period.

Risks:

Besides the possibility of some slight staining of the teeth and a metallic taste, immediately after the irrigation, no side effects have been reported after treatment by SnF_2. The possible stains can be easily removed.

Benefits:

The gingival inflammation may be decreased by this treatment. This means reduced bleeding from the gums and an improved possibility for the dentist to diagnose and treat the disease. Furthermore, it has been well established the fluoride solution used is beneficial in reducing dental caries. To compensate you for your time involved in this study we will offer you preventive dental services (dental cleanings, and fluoride treatments) at the end of the study at no expense, up to $50.00, additional preventive treatment will be billed to the patient.

Figure 5: Human consent form for irrigating SnF_2 into periodontally diseased pockets.

18

Naturally you will have the right to withdraw from this study at any time you wish. Furthermore, the investigators will be available to answer any questions you have during the course of the study.

IT IS NOT THE PRESENT POLICY OF THE UNIVERSITY OF CONNECTICUT TO COMPENSATE HUMAN SUBJECTS IN THE EVENT THE RESEARCH RESULTS IN PHYSICAL INJURY EXCEPT THAT IN FULFILLING ITS PUBLIC RESPONSIBILITY, THE UNIVERSITY OF CONNECTICUT HEALTH CENTER/JOHN DEMPSEY HOSPITAL PROVIDES PROFESSIONAL LIABILITY COVERAGE FOR ANY INJURY IN THE EVENT SUCH INJURY IS CAUSED BY THE FAULT OF THE UNIVERSITY OF CONNECTICUT HEALTH CENTER/JOHN DEMPSEY HOSPITAL. THE UNIVERSITY OF CONNECTICUT HEALTH CENTER/JOHN DEMPSEY HOSPITAL WILL HAVE AVAILABLE THE FACILITIES AND PROFESSIONAL ATTENTION TO CARE FOR SUBJECTS WHO MAY SUFFER A PHYSICAL INJURY AS A RESULT OF PARTICIPATION IN THIS PROJECT. IN THE EVENT THAT YOU HAVE SUFFERED ANY PHYSICAL INJURY AS THE RESULT OF YOUR PARTICIPATION IN THE RESEARCH PROGRAM, PLEASE CONTACT Mrs. Jane Johnson, phone number: 674-2142, WHO CAN REVIEW THE MATTER WITH YOU, IDENTIFY OTHER RESOURCES THAT MAY BE AVAILABLE TO PROVIDE YOU WITH FURTHER INFORMATION AS TO HOW TO PROCEED.

I, THE UNDERSIGNED, HAVE UNDERSTOOD THE ABOVE EXPLANATION AND GIVE CONSENT TO MY VOLUNTARY PARTICIPATION IN DR. TINANOFF'S RESEARCH PROJECT.

DATE:

LOCATION:

_____
SIGNATURE OF THE SUBJECT

_____
WITNESS

_____
DATE

19

The Microbiologic and Clinical Effect of SnF_2 Mouthrinse on a Group of Rampant Caries Adults

Stannous fluoride mouthrinse has been tested in a few short-term studies to see its effect on both the quality and quantity on oral bacteria. These studies have suggested that SnF_2 reduces the number of bacteria in plaque and in saliva, and some studies suggest that this rinse may also be selective against certain oral strains.

The present study was conducted to examine the effect of SnF_2 vs. NaF mouthrinse on a group of adults with high caries activity. This population resembles that which is often seen in the Army. Besides examining the effect of these 2 mouthrinses on plaque and gingivitis, this study also examined the effectiveness of the mouthrinses against certain oral bacteria and against formation of new caries.

This report describes the results from the first six months of a 2-year clinical trial.

Methods and Materials

Patients were selected if they had a high number of S. mutans (greater than 2×10^5/ml saliva) and had large numbers of unrestored carious lesions. Of the 36 patients who started the study, only 18 have been compliant with the required mouthrinse regimen. We defined compliant patients as those who reported no more that 4 missed mouthrinses each month and we verified these reports by monthly monitoring of each patients' remaining supply of mouthrinse.

At baseline, we measured plaque, gingivitis, and DMF besides taking saliva to measure total colony forming units, S. mutans, and lactobacillus per ml saliva. After the measurements, each subject was assigned to the SnF_2 or acidulated sodium fluoride mouthrinse group. Each subject was instructed to rinse twice a day with 10 ml of the respective fluoride

mouthrinse (both at 200 ppm F). Each patient during the course of the study also had all active carious lesions restored and also was placed on a program of oral hygiene instruction, diet counselling, and prophylaxis after the first month of the study.

Follow-up microbial analyses and clinical scorings were performed at 1, 3 and 6 months.

Results

Figure 6 displays the percentage of plaque-free sites in those subjects rinsing with acidulated sodium fluoride. The percentage of plaque-free sites was determined by dividing the number of plaque-free sites by the total number of sites per subject. This graph and subsequent graphs display each subjects' longitudinal change during the study, and we identify each subject by number (right margin). Note in the group rinsing with acidulated sodium fluoride that there is a large general increase in plaque-free sites between the first and third month. This change could be due to the hygiene visits between the first and third month.

Likewise, in the group rinsing with SnF_2 (Figure 7), the increase in plaque-free sites can be attributed to increased oral hygiene. Comparing the SnF_2 group to the acidulated sodium fluoride group, there is no significant difference in the amount of this change between the first and third month. However, cross-sectional analysis of these groups at one month show that the SnF_2 group had a 25% greater increase in plaque-free sites than the acidulated sodium fluoride group during the first month of the trial.

Gingival Index Scores were converted to percentage of non-bleeding sites, or Percentage Gingival Health (Figures 8 and 9) by dividing the frequency of Löe and Silness 0 and 1 scores by the total number of sites per subject. By this measure, gingival health was not markedly increased in the 6 months among subjects rinsing with acidulated sodium fluoride (Figure 8).

21

Most subjects in this group showed little change; one subject showed a great increase in gingival health; others showed a decline in gingival health.

The subjects rinsing with SnF_2, on the other hand, uniformly showed improvement (Figure 9). At 3 months, the variance in this group was very small and only one subject showed a decrease of score between 1 and 3 months.

Comparison of the 2 groups regarding changes in gingival health between 1 and 3 months showed that 8 of 9 subjects rinsing with SnF_2 showed improved gingival health; in the acidulated sodium fluoride group, only 4 of the 9 subjects improved. Because of the unequal variances between groups in gingival scores, analysis of variance could not be used. A t-test of these proportions showed the difference between groups to be statistically significant. The reasons for this adjunctive effect of SnF_2 in the early phase of the study needs further investigation. It does seem clear, however, that in our study of rampant caries adults, twice daily rinses with SnF_2 have a short-term therapeutic effect on gingivitis.

As a further step in our data analysis, we correlated plaque and gingival scores in both groups. The correlation in the group rinsing with acidulated sodium fluoride had an r value of .69, confirming many other reports that quantity of plaque relates to severity of gingivitis. However, the correlation in the SnF_2 group was .34, suggesting that visual plaque deposits may have fewer bacteria or be more pellicle-like. Several other studies have reported increased amounts of pellicle in subjects rinsing with SnF_2.

The longitudinal progress of each subject rinsing with acidulated sodium fluoride with regard to total colony forming units, showed a mean decrease, but not statistically significant, in the total C.F.U. in the course of the study (Figure 10).

The SnF_2 groups also showed a reduction in total colony forming units, which was most evident at the 3 month exam (Figure 11). At three months,

22

there was a 66% reduction in total bacteria from baseline. Between APF and
SnF_2 groups, there was 2.4 x fewer bacteria/ml saliva at 3 months in the
group rinsing with SnF_2 (Table 9).

With regard to S. mutans, the subjects rinsing with APF showed a mean
increase in the S. mutans/ml saliva which at 1 month was significant at
less than .05 (Figure 12). Increase in S. mutans due to APF or NaF has
been reported previously. This somewhat surprising concept may possibly be
explained by previous hypotheses relating increased number of microorganisms
in the presence of fluoride. Either fluoride ions at low concentrations
could stimulate growth of specific microorganisms--in this case S. mutans,
or fluoride could inhibit the general plaque flora allowing reservoirs of
S. mutans in carious lesions to grow out and recolonize the surface.

The SnF_2 rinsing subjects, on the other hand, had greatly reduced
numbers of S. mutans and this organism was almost eliminated in several
subjects at 3 months (Figure 12). At the 6 month scoring, S. mutans appears
in a log scale to increase in most subjects; however, the numerical mean
increase is insignificant, that is, the mean S. mutans count was .2 x 10^6
at 3 months and .3 x 10^6 at 6 months. Preliminary analysis of the 1 year
results show no increase in S. mutans from that found at 6 months.

Comparison of the 2 rinse groups with regard to S. mutans shows dramatic
differences throughout the 6 months. Because the APF group increased
slightly and the SnF_2 group decreased dramatically, the difference between
groups during these 6 months is over 20 x (Table 10). This corresponds to
approximately a 95% difference in S. mutans between groups over the 6 months.
Preliminary analysis of the one year results shows an 18 x difference between
groups.

The number of lactobacillus in the acidulated sodium fluoride group
showed large variability among subjects and within each subject at different

23

examination points. No pattern of change was evident (Figure 13).

The number of lactobacillus in the SnF_2 groups also was quite variable (Figure 14). Even though lactobacillus counts are easily obtainable and accurately read, this study has found that there was great variabilities in lactobacillus counts in individuals over time and no pattern of change was evident due to oral hygiene treatment, restoration of carious lesions, or due to differences in fluoride mouthrinses.

With regard to caries scores, so far not all the 1 year data is in but clearly we see that both groups still continue to have new caries. The subjects rinsing with APF were found to have 4.2 new lesions in 1 year (Table 11), while the SnF_2 group was found to have 2.3 new lesions (Table 12). The small groups in this study may preclude meaningful caries assessment.
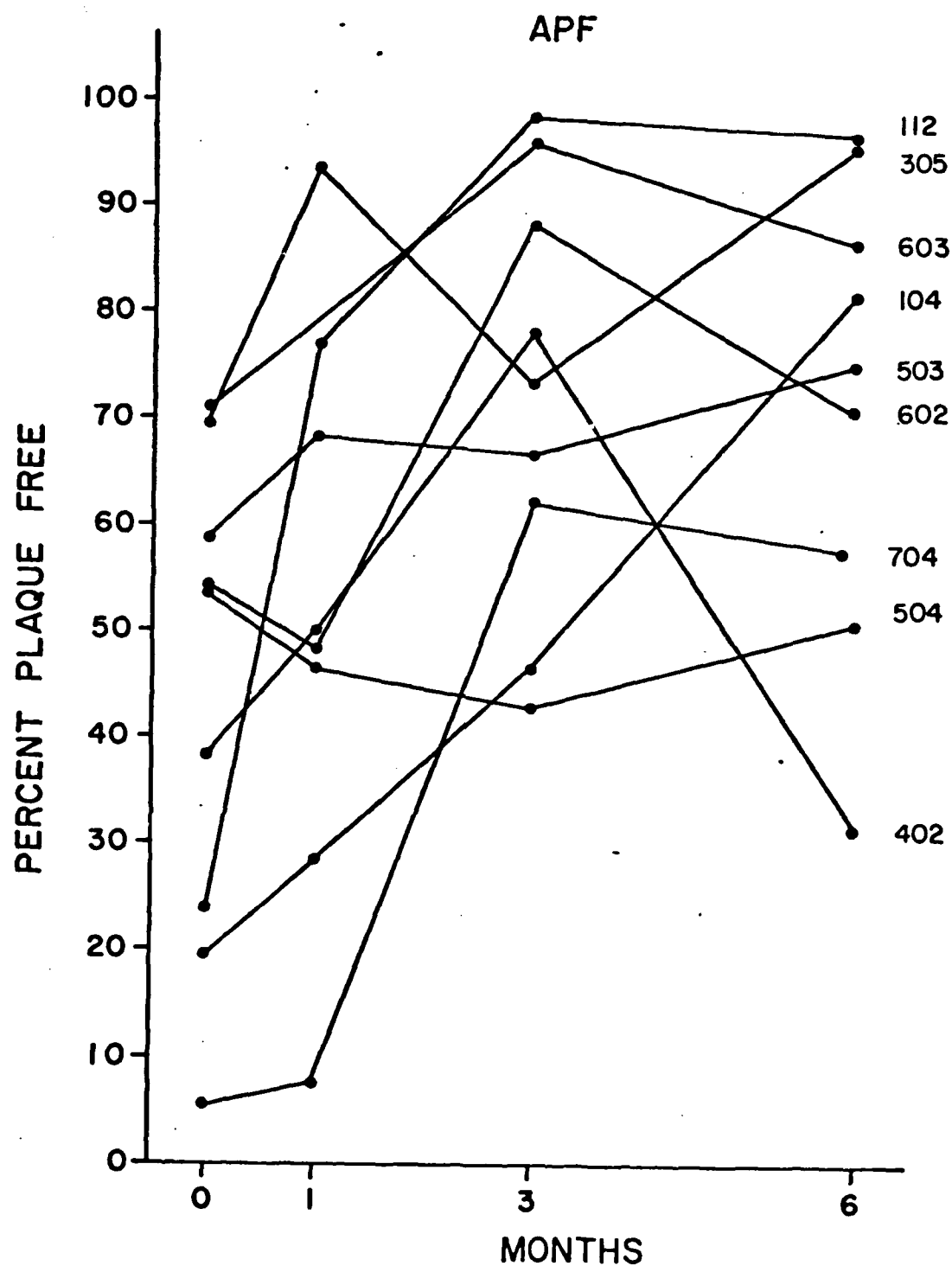
Figure 6: Percentage of plaque-free sites in those subjects rinsing with acidulated sodium fluoride in the first 6 months of the trial.
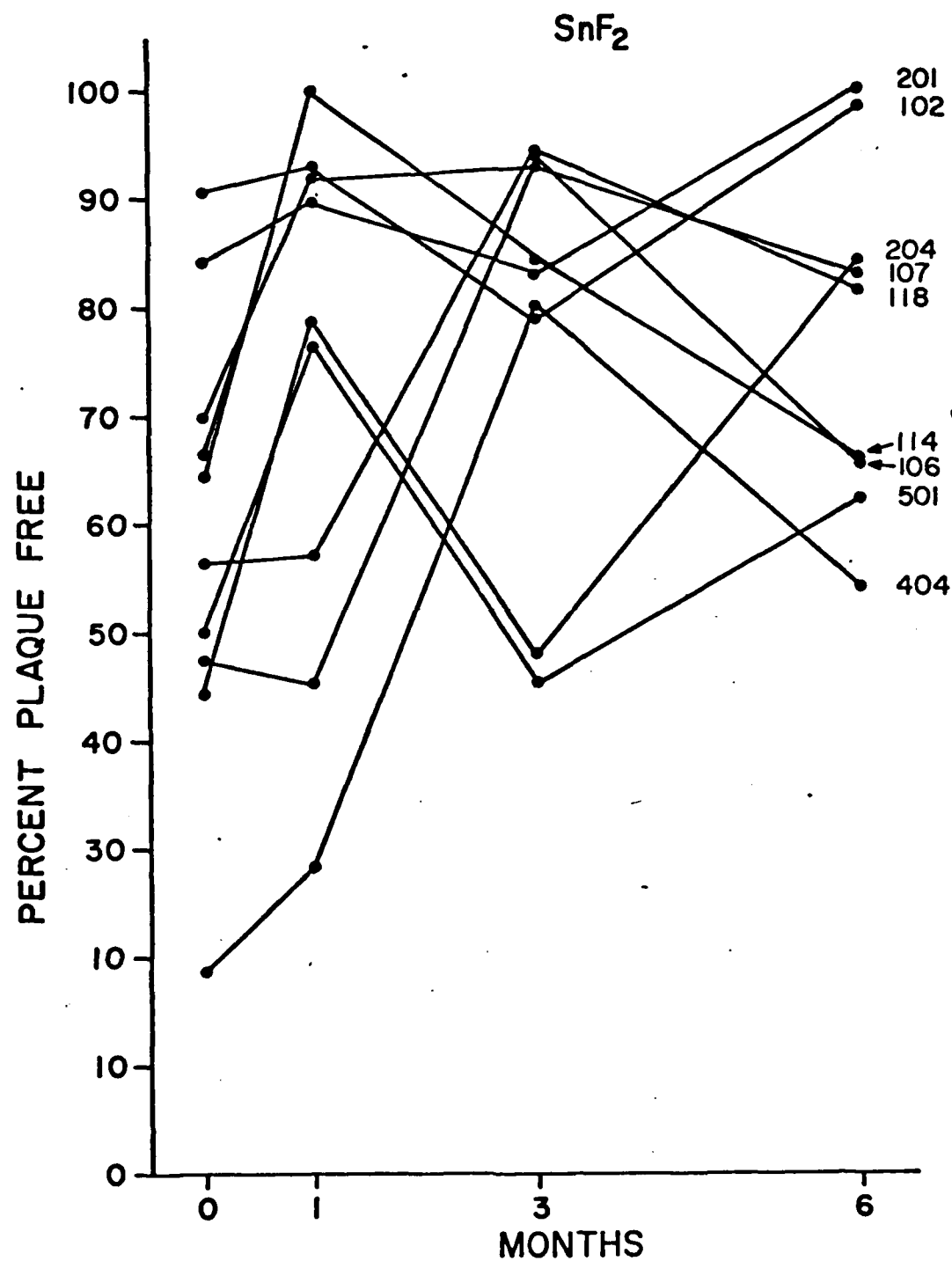
Figure 7: Percentage of plaque-free sites in those subjects rinsing with SnF$_2$ in the first 6 months of the trial.
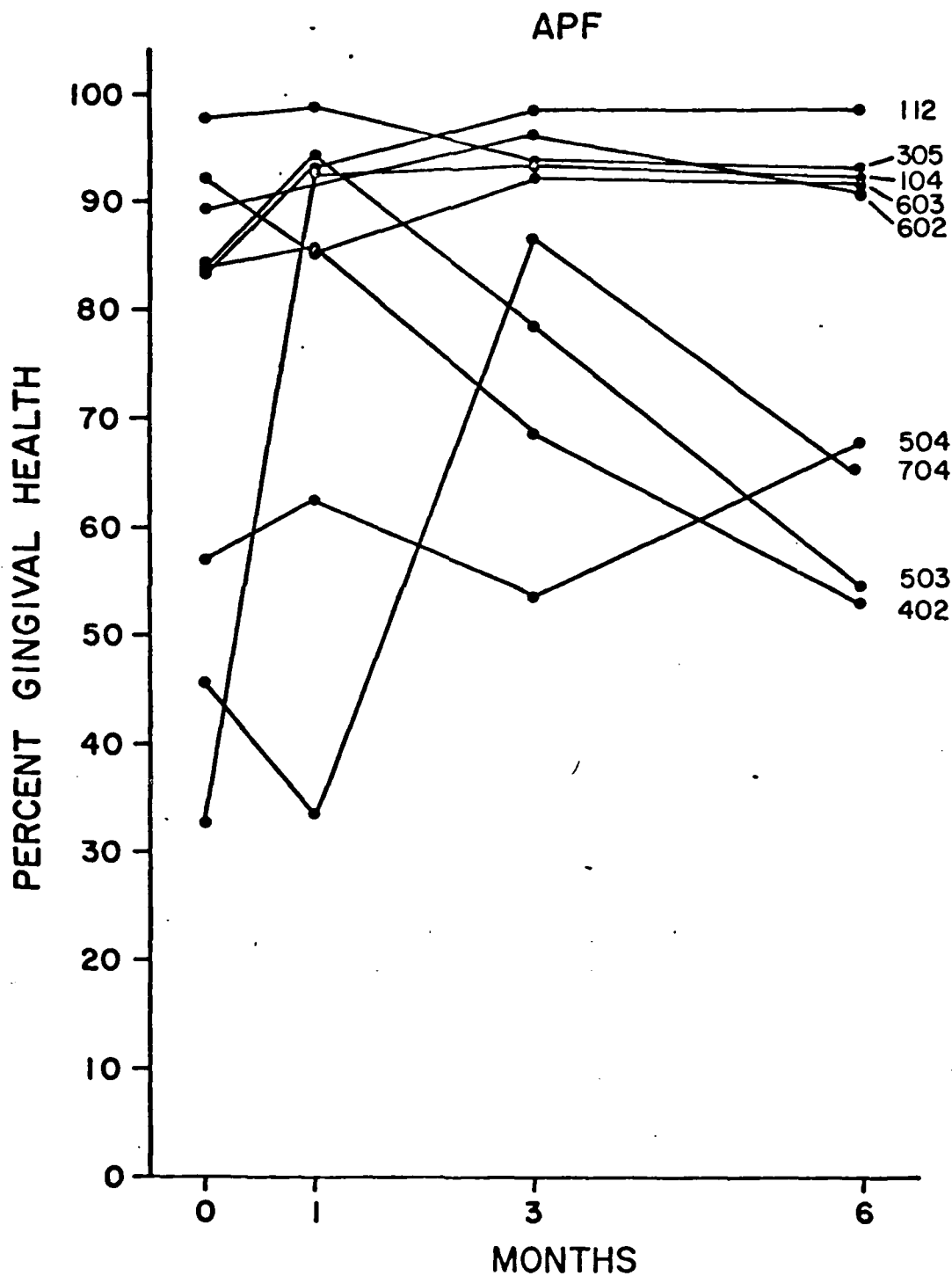
Figure 8: The percentage of gingival healthy sites (G.I. scores of 1 or 2) per subject rinsing with acidulated sodium fluoride over the 6 months trial.
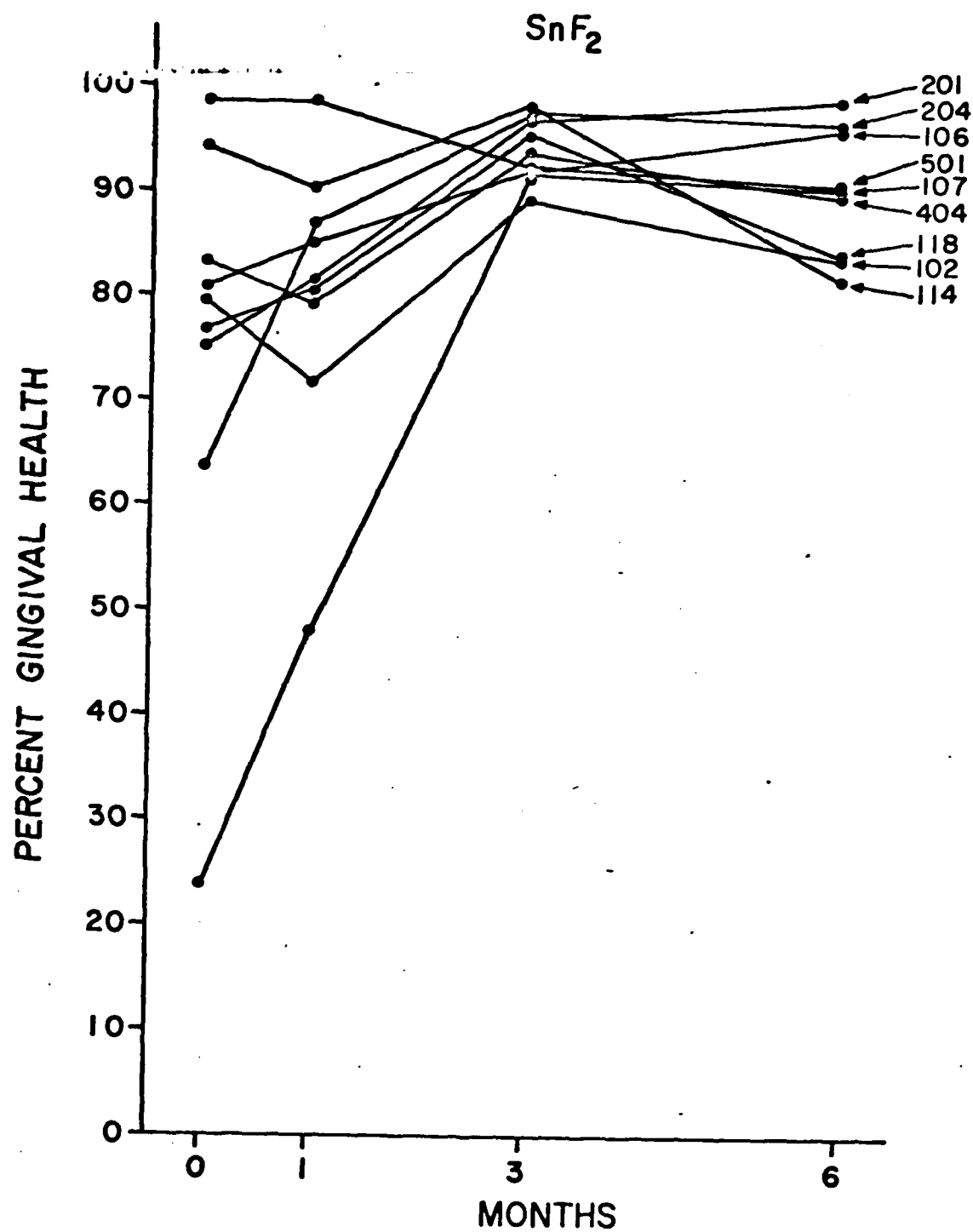
Figure 9: The percentage of gingival healthy sites per subject rinsing with SnF_2 over the 6 month trial.
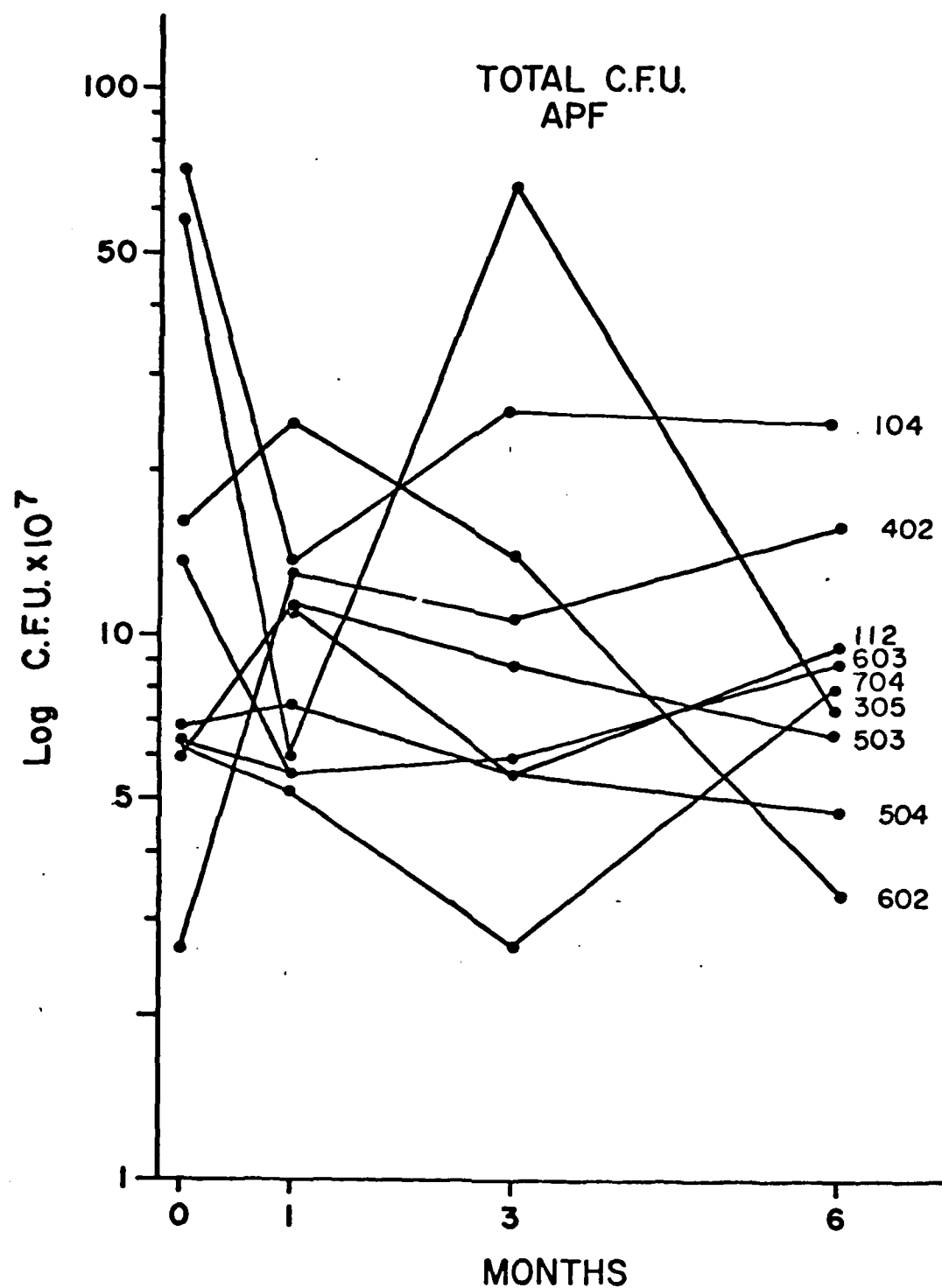
Figure 10: The number of total colony forming bacteria/ml saliva in those subjects rinsing with acidulated sodium fluoride over the 6 month trial.
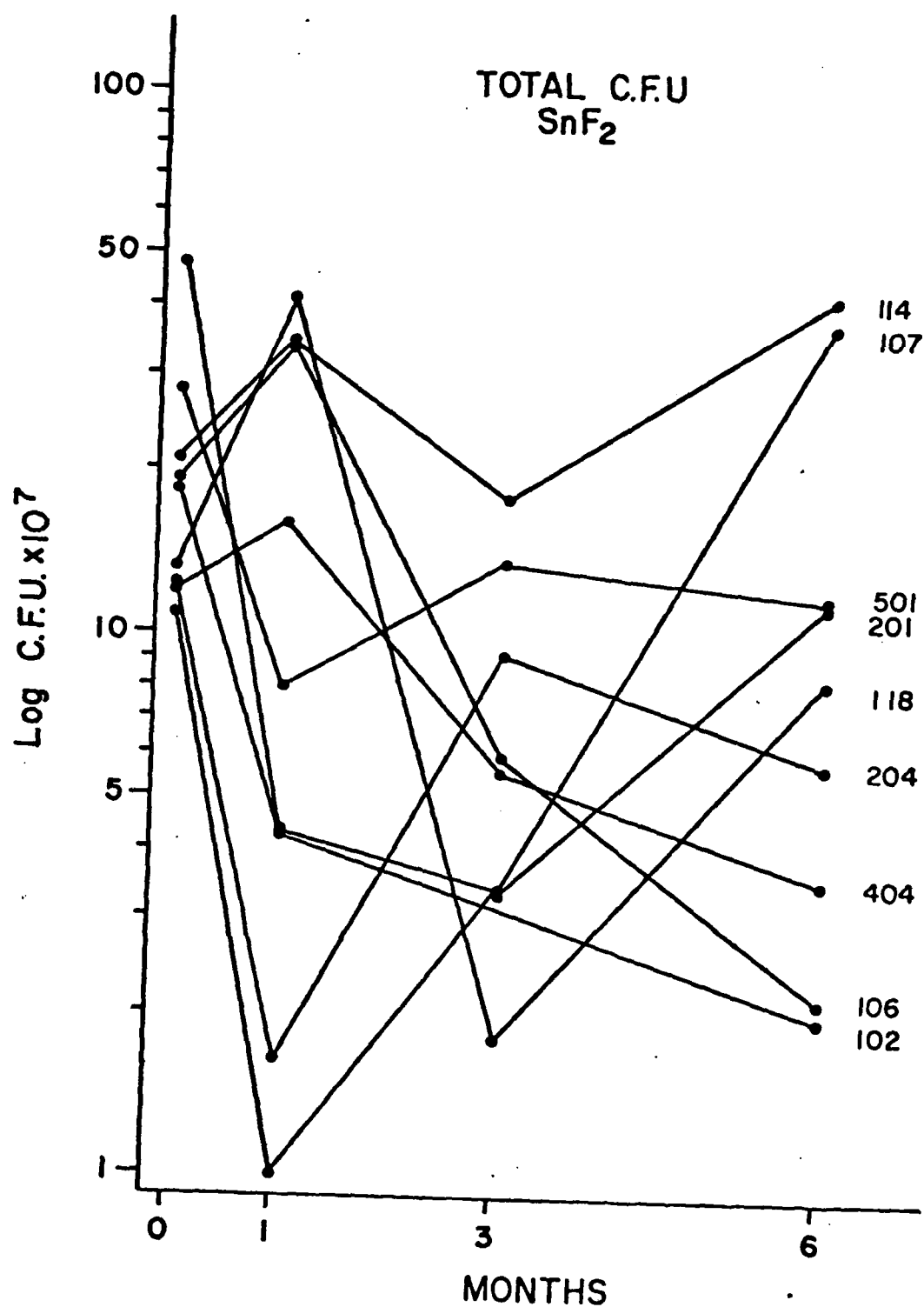
Figure 11: The number of total colony forming bacteria/ml saliva in those subjects rinsing with SnF$_2$ over the six month trial.

Change in Total C.F.U./ml Saliva in Mouthrinse Groups Over 6 Months

	Agent	N	Baseline	1 Month	3 Months	6 Months
C.F.U.	APF	9	23.2 ± 24.9	11.0 ± 6.1	16.3 ± 20.1	10.0 ± 6.6
$x10^6$	SnF_2	9	20.2 ± 11.7	16.2 ± 16.3	6.7 ± 4.1	14.0 ± 15.3

Reduction	Times			1.4x	2.4x	1.4x
	Percent			32%	58%	29%
Significance					>.1	

Table 9: Comparison of the CFU/ml saliva in subjects rinsing with APF vs. SnF_2.
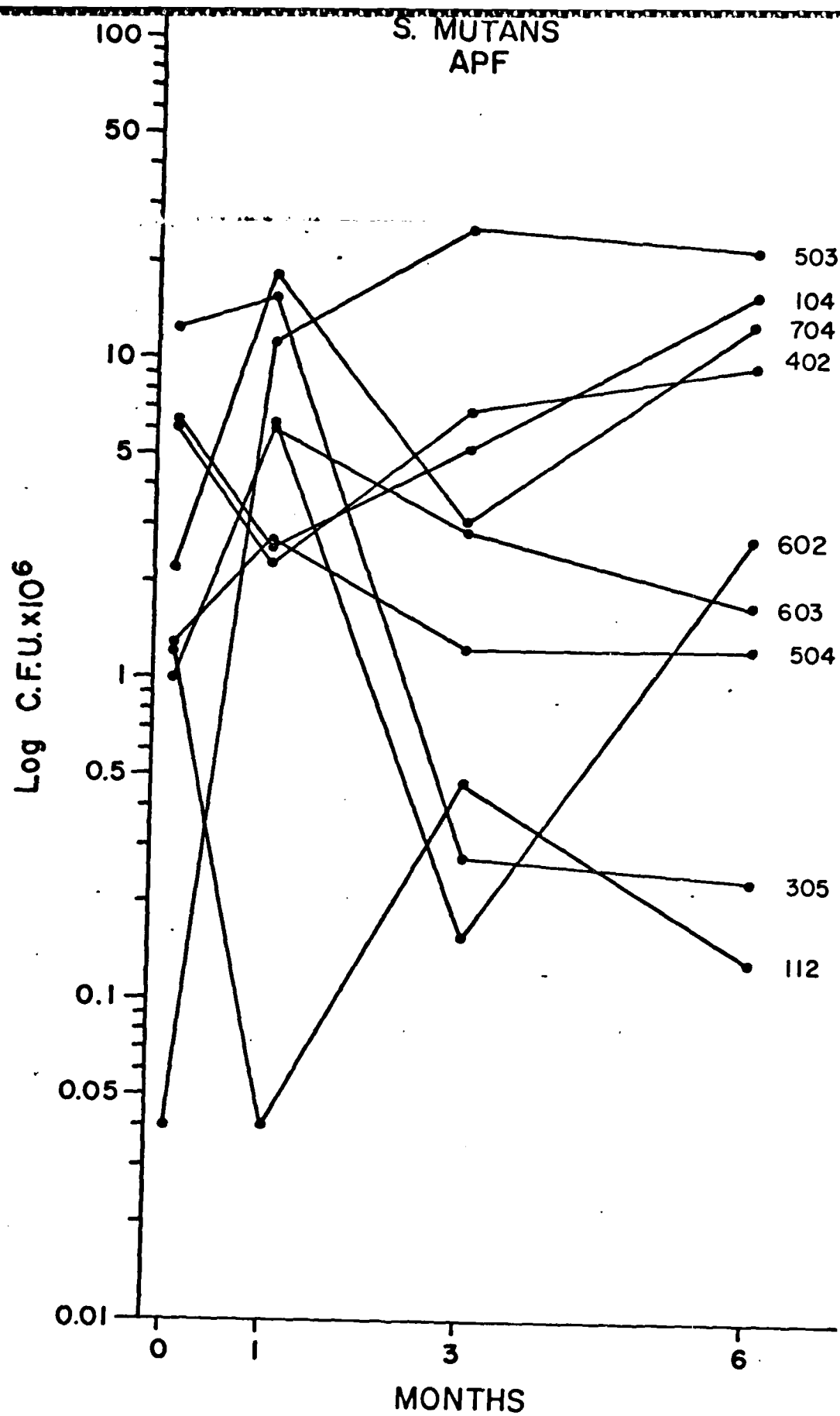
Figure 11: The number of S. mutans/ml saliva in those subjects rinsing with acidulated sodium fluoride over the 6 month trial.
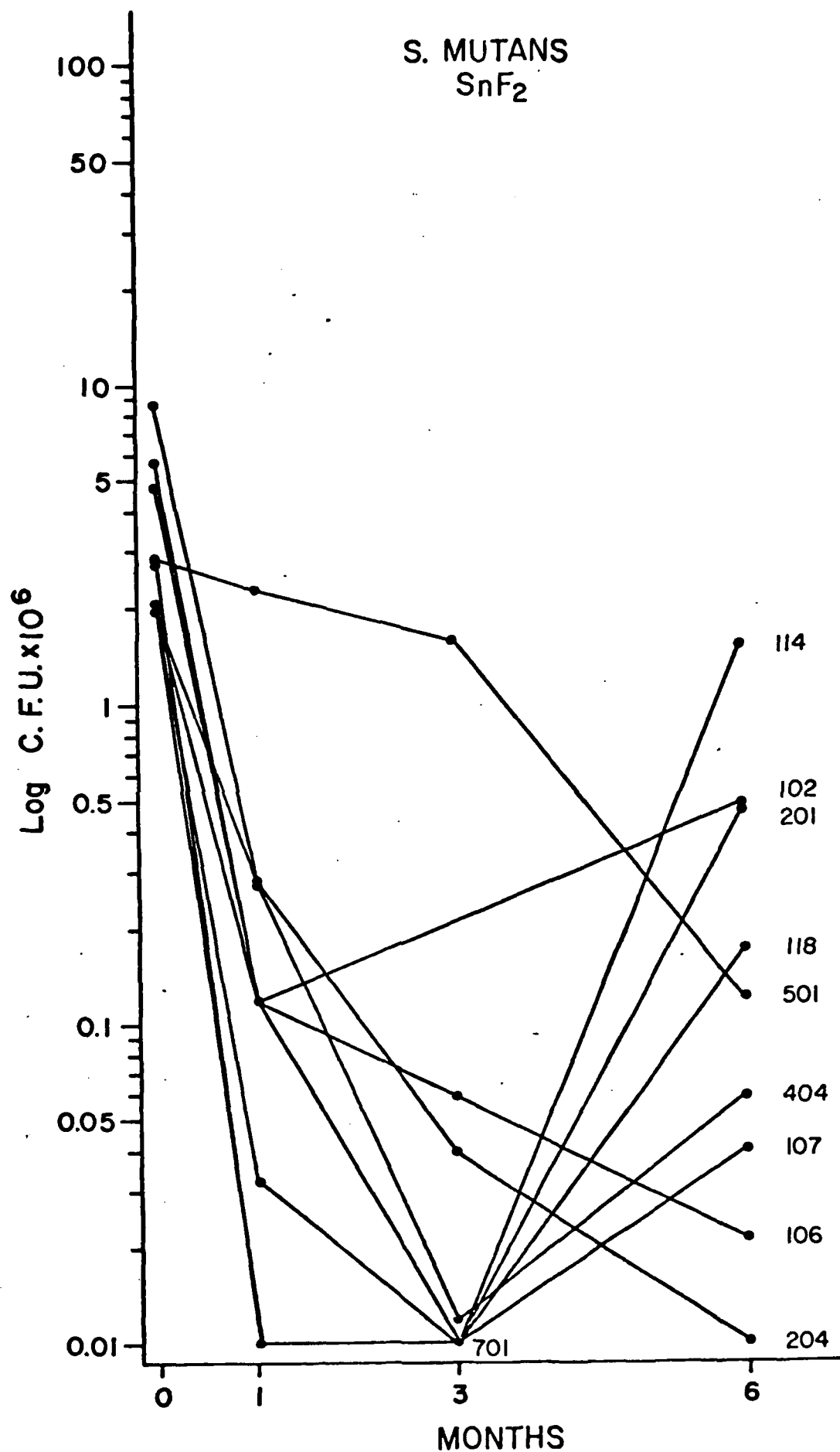
Figure 12: The number of S. mutans/ml saliva in those subjects rinsing with SnF$_2$ over the 6 month trial.

Change in $\underline{S}$. $\underline{mutans}$/ml Saliva in Mouthrinse Groups Over 6 Months

	Agent	$\underline{N}$	Baseline	1 Month	3 Months	6 Months	Mn
C.F.U.	APF	9	3.2 ± 3.5	7.2 ± 6.3	5.2 ± 7.8	7.7 ± 7.5	5.5±4.?
x10^6	SnF$_2$	9	3.7 ± 2.3	0.4 ± 0.7	0.2 ± 0.5	0.3 ± 0.5	0.3 ±.38
Reduction	Times			18x	26x	26x	1?x
	Percent			94%	96%	96%	
Significance				>.05	>.05	>.05	

Table 10: Comparison of $\underline{S}$. $\underline{mutans}$/ml saliva in those subjects rinsing with APF vs. SnF$_2$ over the 6 months.
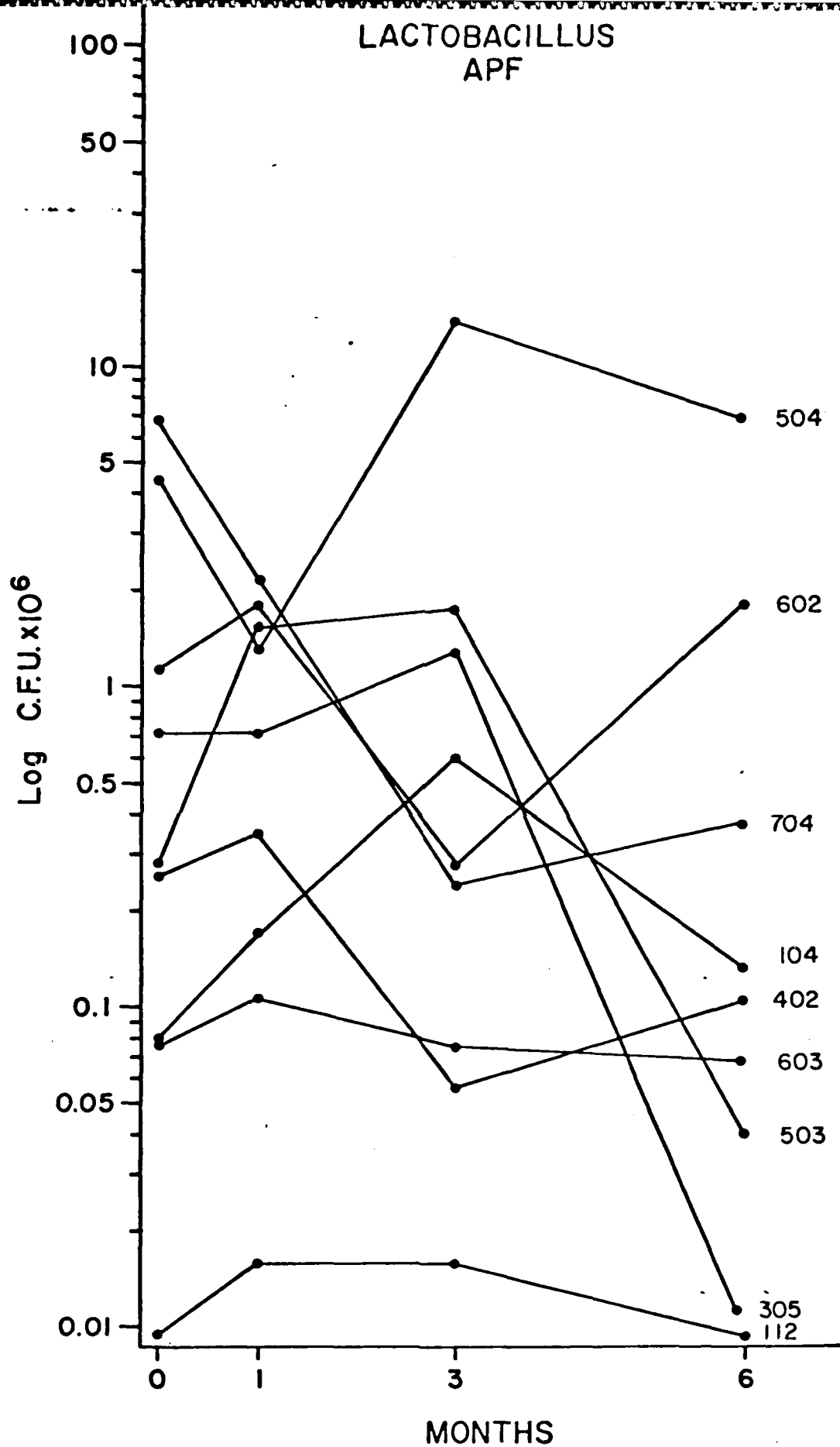
Figure 13: The number of lactobacillus/ml saliva in those subjects rinsing with acidulated sodium fluoride over 6 monts.
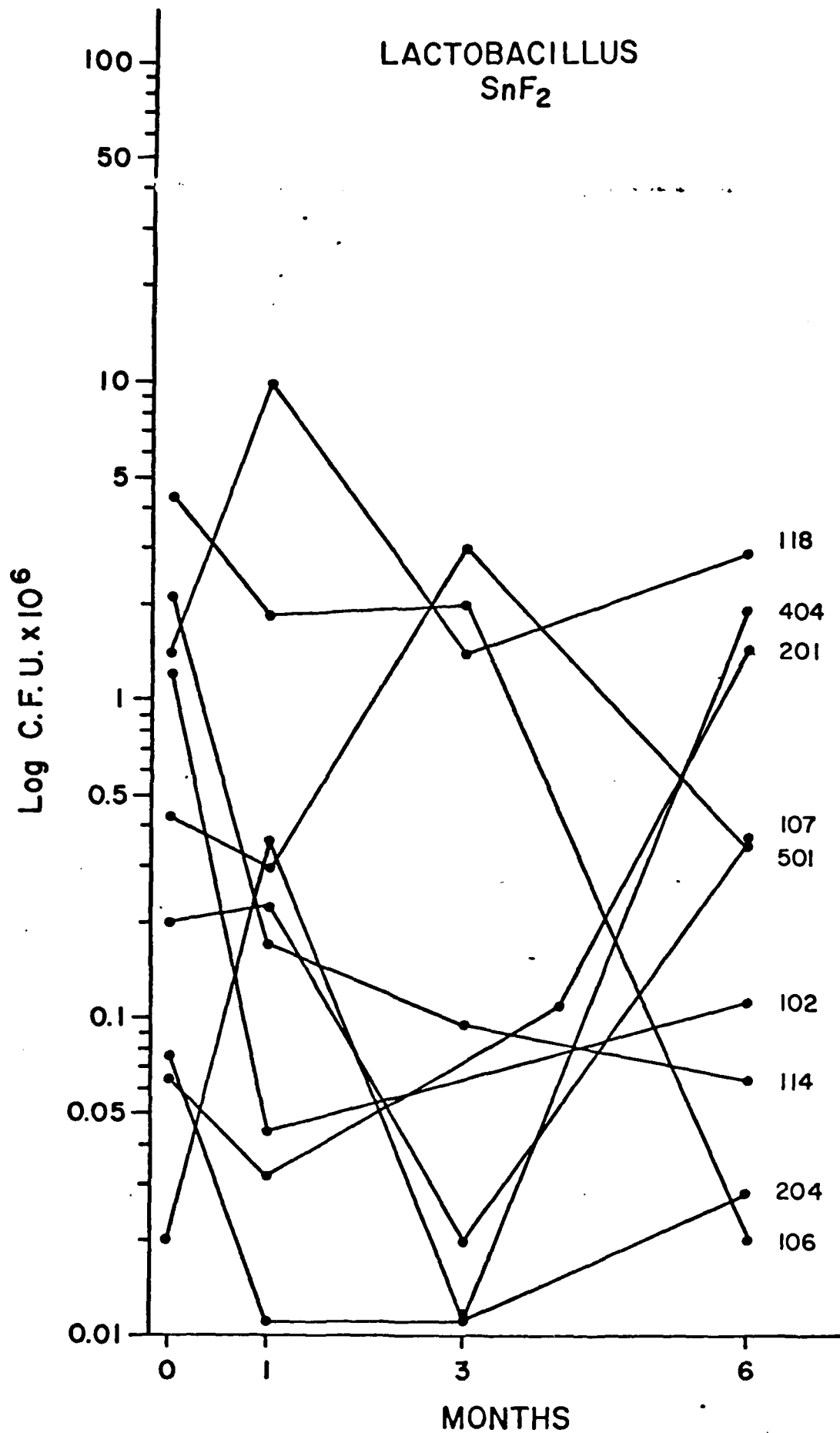
Figure 14:   The number of lactobacillus/ml saliva in those subjects rinsing with SnF$_2$ over the 6 month trial.

Compliant

Subject	Initial White Spots	D	M	F (S)	New Lesions	Recurrent Decay	Root Caries	Σ
104	8	13-	24-	31	1			1
112	6	7-	4-	0	7 (5 occ)			
305	4	19-	25-	6	4			4
503	10	21-	29-	40	2			2
504	8	12-	0-	85	3 (1 occ)	4		7

$\overline{x}$ 4.2

Non-Compliant

Subject	Initial White Spots	D	M	F (S)	New Lesions	Recurrent Decay	Root Caries	Σ
203	15	24-	15-	9	7			7
316	3	11-	50-	4		1		1

Total $\overline{x}$ 4.1

Table 11: Initial caries and new caries after 1 year in those subjects rinsing with acidulated sodium fluoride.

SnF_2

Compliant

Subject	Initial White Spots	D	M	F	(S)	New Lesions	Recurrent Decay	Root Caries	Σ
102	11	5-	0-	28		2			2
106	5	4-	35-	34		2		1	3
107	2	24-	22-	29		2			2
114	12	13-	15-	32					0
118	14	7-	0-	69			5		5
701	12	36-	39-	10					0
704	12	25-	14-	51		4 (1 occ)			4

$\overline{x}$ 2.3

Non-compliant

Subject	Initial White Spots	D	M	F	(S)	New Lesions	Recurrent Decay	Root Caries	Σ
103	6	6-	15-	59		5			5
303	5	2-	15-	86		1			2
307	2	16-	35-	40		1	1		2
401	12	5-	42-	31			1		1
404	1	9-	30-	34		3	1		4

Total $\overline{x}$ 2.5

Table 12: Initial caries and new caries after 1 year in those subjects rinsing with SnF_2.

Appendix A

List of Publications and Presentations Supported by
U.S. Army Contract DAMD 17-81-C-1075

Papers in press:

*1. Ferretti, G. A., Tanzer, J. M. and Tinanoff, N. The Effect of
     Fluoride and Tin on S. mutans Viability, Growth, Acid and
     Glucan Production. Caries Research

*2. Swanson, T. D. and Tinanoff, N. Antiplaque Properties of Sustained
     Release SnF_2. Journal of Oral Rehabilitation

Papers in preparation:

1. Camosci, D. A. and Tinanoff, N. Antiplaque Determinants of SnF_2:
    pH and Ions. Antimicrob. Agents. Chemother.

2. Ferretti, G. A. and Tinanoff, N. Ultrastructure of S. mutans Grown
    in Media Supplemented with Fluoride and Tin. Caries Research

3. Tinanoff, N., Serling, J. M., Manwell, M. A., Clive, J. M. Effect
    of NaF vs. SnF_2 Mouthrinses on Gingivitis and Plaque. Journal
    of Periodontology

4. Tinanoff, N., Manwell, M. A., Camosci, D. A. Effect of NaF vs.
    SnF_2 Mouthrinses on the Salivary Flora. Journal of Dental Research

5. Klock, B. and Tinanoff, N. Effect of NaF vs. SnF_2 Mouthrinses on
    Caries Progression. Scandinavian Journal of Dental Research

Published Abstracts:

*1. Camosci, D. A. and Tinanoff, N. Effect of pH on SnF_2 Stability and
     Antiplaque Properties. Proc. Int. Assoc. Dent. Res., New Orleans,
     March, 1982.

*2. Serling, J. M., Clive, J. M., Manwell, M. A., Tinanoff, N. Effect
     of NaF vs. SnF_2 Mouthrinse on Gingivitis and Plaque - 6 Months
     Results. Proc. Int. Assoc. Dent. Res., New Orleans, March,
     1982.

*3. Tinanoff, N., Manwell, M. A., Camosci, D.A., Klock, B. The Micro-
     biologic Effect of SnF_2 vs NaF Mouthrinse After 6 Months. Proc.
     Int. Assoc. Dent. Res., New Orleans, March, 1982.

*Paper attached in appendix.

Presentations:

1. Tinanoff, N. Antiplaque Properties of SnF_2 Mouthrinse. Research
   Program, Annual Meeting of American Academy of Pedodontics,
   May 26, 1981.

2. The Antiplaque Effects of SnF_2. National Naval Dental Center
   course in Preventive Dentistry and Patient Motivation, Washington,
   D.C., December 7-10, 1981.

3. Antiplaque Effects of SnF_2. American Society of Dentistry for
   Children, Maryland Component, Baltimore, December 10, 1981.

Appendix B

Published Abstracts Supported by

U.S. Army Contract DAMD 17-81-C-1075

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

ABSTRACT FORM

Complete: Items 1-8 below and type abstract within box, following instructions on the reverse side.

Submit: Abstract form—Original and 3 Xerox copies. Complete presenter card (white) listing all co-authors.

for: (Check one)
- ☒ IADR MEETING
- ☐ AADR MEETING
- ☐ _____ DIVISION MEETING

1. DEADLINE FOR SUBMISSION: OCTOBER 12, 1981

2. CITY OF MEETING: NEW ORLEANS, LA

3. DATES OF MEETING: MARCH 18-21, 1982

4. Complete name and mailing address of the **PRESENTER**

David A. Camosci
Department of Pediatric Dentistry
Univ. of Conn. Health Center
Farmington, CT 06032

IADR member? (1) ☐ yes (2) ☒ no

5 Mode of presentation:
(check only one)
(1) ☒ oral presentation only
(2) ☐ poster presentation only
(3) ☐ oral or poster acceptable

6 Do you wish to withdraw your paper if it is placed in a mode not of your choosing?
(1) ☒ yes   (2) ☐ no

7. Would you accept "read by title" as an alternative to withdrawal?
(1) ☒ yes   (2) ☐ no

8 Group Classification: (check one)
(a) Behavioral Science
(b) Cariology
(c) Craniofacial Biology
(d) Dental Materials
(e) Experimental Pathology
(f) Microbiology Immunology
(g) Mineralized Tissue
(h) Neuroscience
(i) Oral & Maxillofacial Surgery
(j) Periodontal Research
(k) X Pharmacology, Therapeutics & Toxicology
(l) Prosthodontics Research
(m) Pulp Biology
(n) Salivary Research
(o) Other _____

42

TYPE PERFECT ORIGINAL OF ABSTRACT HERE
(Do not type beyond outline of box.)

Effect of pH on SnF_2 Stability and Anti-plaque Properties. D.A. CAMOSCI* and N. TINANOFF, UConn School of Dental Medicine, Farmington, CT.

Aqueous solutions of SnF_2, while having antiplaque properties, are known to be unstable. The purpose of these experiments was to test in vitro the stability and antiplaque properties of aqueous solutions of SnF_2 (250 ppm F^-) at various pH's. Furthermore, the pH of commercial preparations of SnF_2 were measured prior to and after its use as a mouthrinse. In vitro tests consisted of growth of S. mutans on wires with exposure every 12 h for 1 min to either SnF_2 pH 2, 3, 4, 5, 6; NaF pH2 and 6; or H_2O pH 2 and 6. After 48 h, plaque thickness on the wire was scored, plaque dry weight was determined, and Sn/mg plaque measured by AAS. For in vivo tests, commercial preparations of SnF_2 (0.4%) were prepared to 250 ppm F^- by diluting with deionized H_2O (pH 6.9). The pH's of these preparations were measured prior to and after a 1 min mouthrinse in 5 subjects.

In vitro, lower plaque scores were noted for all samples exposed to SnF_2 when compared to NaF and H_2O; yet above pH 4.5, SnF_2 became less effective as an antiplaque agent. An inverse relationship existed for plaque weight vs. Sn/mg plaque suggesting that tin accumulation in bacteria correlated to the antiplaque properties of SnF_2. In vivo, SnF_2 preparations were noted to be below pH 4.1 prior to rinsing, and one initially at pH 3.3 rose to a mean pH of 4.4 after the 1 min mouthrinse. The antiplaque properties of SnF_2 could not be attributed to pH or HF effects on the bacteria. Loss of antiplaque properties of SnF_2 was a result of tin precipitation. The in vivo trial suggested salivary buffering does not raise the pH of SnF_2 to an ineffective level.

9. Reviewer's Ratings:
[ ] [ ] [ ] [ ] [ ]
 1   2   3   4   5

10. Disposition:
[ ] [ ] [ ] [ ] [ ]
 O .P   T   R   W

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

ABSTRACT FORM

Complete: Items 1-8 below and type abstract within box, following instructions on the reverse side.

Submit: Abstract form—Original and 3 Xerox copies. Complete presenter card (white) listing all co-authors.

for: (Check one)

[ ] IADR MEETING

[ ] AADR MEETING

[ ] _____ DIVISION MEETING

1. DEADLINE FOR SUBMISSION: OCTOBER 12, 1981

2. CITY OF MEETING: NEW ORLEANS, LA

3. DATES OF MEETING: MARCH 18-21, 1982

4. Complete name and mailing address of the **PRESENTER**

Dr. J.M Serling
Dept. of Periodontics
School of Dental Medicine
Univ. of Conn. Hlth. Ctr.
Farmington, Ct. 06032

IADR member? (1) [X] yes (2) [ ] no

5. Mode of presentation:
(check only one)
(1) [X] oral presentation only
(2) [ ] poster presentation only
(3) [ ] oral or poster acceptable

6. Do you wish to withdraw your paper if it is placed in a mode not of your choosing?
(1) [X] yes   (2) [ ] no

7. Would you accept "read by title" as an alternative to withdrawal?
(1) [X] yes   (2) [ ] no

8. Group Classification: (check one)
(a)   Behavioral Science
(b)   Cariology
(c)   Craniofacial Biology
(d)   Dental Materials
(e)   Experimental Pathology
(f)   Microbiology Immunology
(g)   Mineralized Tissue
(h)   Neuroscience
(i)   Oral & Maxillofacial Surgery
(j)   Periodontal Research
(k) X Pharmacology, Therapeutics & Toxicology
(l)   Prosthodontics Research
(m)   Pulp Biology
(n)   Salivary Research
(o)   Other _____

TYPE PERFECT ORIGINAL OF ABSTRACT HERE
(Do not type beyond outline of box.)

Effect of NaF vs. SnF$_2$ Mouthrinse on Gingivitis and Plaque - 6 Months Results. J.M. SERLING*, J.M. CLIVE, M.A. MANWELL, N. TINANOFF. UConn School of Dental Medicine, Farmington, Ct.

Animal studies have suggested that SnF$_2$ rinses improve gingivitis. Human studies, even though showing antiplaque effects, have not yet demonstrated a beneficial effect on gingivitis. One of the purposes of this 2 year NaF vs. SnF$_2$ mouthrinse study was to examine the influence of these agents on gingivitis.
High caries experience adults who were systematically distributed into 2 groups by S. mutans counts had, at baseline, identical mean gingivitis (GI = 1.22). Both groups rinsed unsupervised 2X/day, one with SnF$_2$, one with APF (200 ppmF$^-$). After baseline scoring, the subjects began rinsing; after the 1 month scoring each subject had 3 visits of oral hygiene instruction and prophylaxis; after the 3 month scoring, OHI and prophylaxis was repeated. Final plaque and gingivitis scores were taken at 6 mo. The frequencies for scores of GI and PlI were calculated for each individual longitudinally as well as cross-sectionally.
The results showed that the change of healthy gingival scores (0 & 1) was not different between groups from baseline to 6 mo.; however, the SnF$_2$ group showed a significant improvement (p<.1) over the APF group between 3 and 6 mo. Plaque scores were not different between groups from baseline to 6 mo., yet when comparing 1 mo. to 6 mo., the SnF$_2$ group was significantly better.
The results suggest that SnF$_2$ may be an adjunct in improving plaque and gingivitis parameters when associated with a program of oral hygiene and professional cleaning.

43

9. Reviewer's Ratings:
[ ][ ][ ][ ][ ]
1 2 3 4 5

10. Disposition:
[ ][ ][ ][ ]
O P T R W

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

ABSTRACT FORM

Complete: Items 1-8 below and type abstract within box, following instructions on the reverse side.

Submit: Abstract form—Original and 3 Xerox copies. Complete presenter card (white) listing all co-authors.

for: (Check one)
- [X] IADR MEETING
- [ ] AADR MEETING
- [ ] _____ DIVISION MEETING

1. DEADLINE FOR SUBMISSION: OCTOBER 12, 1981
2. CITY OF MEETING: NEW ORLEANS, LA
3. DATES OF MEETING: MARCH 18-21, 1982

TYPE PERFECT ORIGINAL OF ABSTRACT HERE
(Do not type beyond outline of box.)

4. Complete name and mailing address of the **PRESENTER**

NORMAN TINANOFF, D.D.S.
DEPT. OF PEDIATRIC DENTISTRY
U. OF CONN. HEALTH CTR.
FARMINGTON, CT ON. 06032

IADR member? (1) [X] yes (2) [ ] no

5. Mode of presentation:
   (check only one)
   (1) x oral presentation only
   (2) i poster presentation only
   (3) oral or poster acceptable

6. Do you wish to withdraw your paper if it is placed in a mode not of your choosing?
   (1) x yes   (2) [ ] no

7. Would you accept "read by title" as an alternative to withdrawal?
   (1) x yes   (2) [ ] no

8. Group Classification: (check one)
   - (a) Behavioral Science
   - (b) Cariology
   - (c) Craniofacial Biology
   - (d) Dental Materials
   - (e) Experimental Pathology
   - (f) Microbiology Immunology
   - (g) Mineralized Tissue
   - (h) Neuroscience
   - (i) Oral & Maxillofacial Surgery
   - (j) Periodontal Research
   - (k) X Pharmacology, Therapeutics & Toxicology
   - (l) Prosthodontics Research
   - (m) Pulp Biology
   - (n) Salivary Research
   - (o) Other _____

The Microbiologic Effect of SnF_2 vs. NaF Mouthrinse After 6 Months. N. TINANOFF,* M.A. MANWELL, D.A. CAMOSCI, B. KLOCK. UConn School of Dental Medicine, Farmington, CT.

Numerous short-term studies have shown reduction of plaque formation by SnF_2 mouthrinse, yet alterations in numbers of potential cariogenic organisms due to this agent has not been done. Different sodium fluoride mouthrinses, on the other hand have not been reported to have any antiplaque effects at mouthrinse concentrations. One of the purposes of this 2 year mouthrinse study is to compare the effect of SnF_2 and NaF of comparable pH's on salivary-total colony forming units (CFU), S. mutans and lactobacilli.

Adults exhibiting high caries experience and having over $2x10^5$ S. mutans/ml saliva were distributed into 2 groups that rinsed unsupervised, 2 times per day with either SnF_2 or APF (200 ppm F^-). Salivary samples were collected at baseline, 1, 3, and 6 months for bacteral quantitation by the method of Westergren and Krasse (1978).

There was a small reduction (2x) in total CFU in both the SnF_2 and APF groups at 1, 3, and 6 mo.; no reduction in lactobacillus in either group; however, S mutans reduction in the SnF_2 group was 5 x at 1 mo., 9 x at 3 mo. and 5 x at 6 mo. from baseline. When the SnF_2 group was compared to the APF group the reductions were 9 x, 12 x, and 10 x for 1, 3, and 6 mo., respectively.

The results support that rinsing with SnF_2 selectively affects S. mutans on a long term basis. One and 2-year caries exams will determine whether the reduction in S. mutans is correlated to the increment of caries.

44

9. Reviewer's Ratings: 1 2 3 4 5

10. Disposition: O P T R W

Appendix C

Papers "In Press" Supported By
U.S. Army Contract DAMD 17-81-C-1075

45

ANTIPLAQUE PROPERTIES OF SUSTAINED RELEASE SnF_2:

PILOT STUDIES

By

T. D. Swanson, D.D.S.

N. Tinanoff, D.D.S., M.S.

Department of Pediatric Dentistry
School of Dental Medicine
University of Connecticut Health Center
Farmington, Connecticut 06032

Running Title: Sustained Release SnF_2

Key Words: SnF_2, antiplaque, controlled release

Communications to: N. Tinanoff, D.D.S., M.S.
                    Department of Pediatric Dentistry
                    School of Dental Medicine
                    University of Connecticut Health Center
                    Farmington, CT. 06032

ABSTRACT

Pilot studies examining the physical and clinical properties of an intracoronal sustained release fluoride delivery system were performed. After testing various percentages of SnF_2 incorporated into polycarboxylate, zinc phosphate, and zinc oxide cements, 70 percent SnF_2 polycarboxylate cement was found to have adequate compressive strength while releasing the greatest amount of fluoride _in vitro_.

A 30 day _in vivo_ trial in which this fluoride-cement was used as a temporary intracoronal restoration produced elevated salivary fluoride levels with only transient elevation in urinary fluoride levels. Plaque scores decreased during the experimental period suggesting that the released SnF_2 affected bacterial growth or attachment. The SnF_2-polycarboxylate cement was an adequate temporary restorative material without significant side effects.

INTRODUCTION

The effective delivery of antimicrobials as well as other chemothera-
peutic agents for the prevention or treatment of bacterial infections of
tooth surfaces may be suboptimal due to its reliance on patient cooperation.
Conventional methods for delivering of these agents to the oral cavity in-
volve use of mouthrinses, gels, and dentifrices (Ainamo, 1977), and these
systems are compromised in varying degrees due to their reliance on patient
cooperation for repeated applications of the chemotherapeutic agent (Mirth
and Bowen, 1976).

Interest in sustained release systems for drug delivery in medicine
and dentistry has been increasing. Besides taking the repeated administra-
tion of a drug away from patient responsibilities, controlling the rate and
site of release may be a more effective means of administering a drug. To
date, sustained release systems in dentistry have been explored for delivery
of steroids for the management of apthous ulcers (Yeoman, Greenspan, and
Harding, 1978); anti-fungal drugs for the management of denture stomatitis
(Douglas and Walker, 1973; Thomas and Nutt, 1978); antibacterials for the
control of plaque (Addy, 1981) and fluorides for the control of dental
caries and remineralization (Mirth and Bowen, 1976; Duperon and Jedrychowski,
1980; Forsten, 1976; Zity, Gedalia, and Grajower, 1981; Whitford et al.,
1980; Friedman, 1980; Mirth et al., 1981; Abrahams et al., 1981). To date,
the largest clinical study has been performed with a trilaminate methacrylate
sodium fluoride-releasing device cemented to the buccal surfaces of the
teeth of 11 subjects. The intraoral device was found to elevate the levels
of fluoride in plaque, saliva and urine, but had no effect on plaque or
gingival parameters (Mirth et al., 1981).

48

Fluoride ions may act as a therapeutic agent by altering bacterial metabolism (Hamilton, 1977) as well as reacting physicochemically with enamel to reduce enamel solubility or remineralize initial caries (for review, see Mellberg, 1976). Yet only stannous fluoride has been shown to reduce the quality of plaque at concentrations compatible with frequent oral use (for review, see Tinanoff and Weeks, 1979). Based on its demonstrated ability to inhibit plaque, stannous fluoride was chosen as the active agent whose effectiveness might best be enhanced by incorporation into a sustained release delivery system. The depot for the SnF_2 was an intracoronal (tooth) preparation where the SnF_2 was mixed with a dental cement and used as a temporary restoration.

The purpose of these pilot studies was to develop a SnF_2 intraoral sustained release delivery system; and to evaluate the delivery system in vivo for antiplaque properties, oral fluoride release, and systemic effects.

METHODS AND MATERIALS

In Vitro Tests

Cement Preparation

To four dental cements--zinc phosphate cement (Improved powder, type I.,
S.S. White), polycarboxylate cement (Durelon, Premier), reinforced zinc oxide-
eugenol (IRM, Caulk) and zinc oxide-eugenol (generic)--stannous fluoride
(Ozark-Mahoning) was added (W/W powder) to produce ratios of 20, 40, and 60
percent. Stannous fluoride was also added to polycarboxylate powder at a
70 percent ratio based on earlier pilot studies. Prior to incorporating the
SnF_2 into the cement, the fluoride crystals were pulverized to a fine powder
by triturating the crystals in an amalgamator (Wiggle-bug LP60, Cresent
Dental) for 1 minute at maximum velocity.

The cements with or without addition of the SnF_2 powder were mixed by
one operator as recommended by the manufacturer; i.e., zinc phosphate was
mixed on a glass slab using incremental additions of powder to liquid over
a 2 minute period; polycarboxylate (Durelon) was mixed on a plastic-coated
paper pad and spatulated for 30 seconds; IRM was mixed on an absorbant paper
pad incrementally and thoroughly spatulated; zinc oxide eugenol was mixed
with the same technique as IRM.

Compressive Strength of Cements

After the appropriate mixing of the cement formulations, each sample
was used to fill three 10 x 20 mm plastic capsules (Beem Capsules, size 00,
Polysciences, Inc.). Following several days to allow for complete set, the
cements were removed from the capsule and the ends ground parallel on a
silicone carbide wheel to a standard height of 7.3 mm. Ultimate compressive
strengths of the dry samples were measured on a materials testing instrument
(Instron, Model 1113) with a crosshead speed of 0.5 cm/min. Some selected

specimens which underwent a 30 day fluoride leaching trial were also tested for post-leaching compressive strengths.

Leaching of Fluoride from Cements

A cylindrical specimen of each fluoride concentration from the four cements was prepared, removed from the mold, and then coated with blue inlay wax (Kerr Products) so that only one open, circular end was exposed. (The poor set and low compressive strength of IRM allowed only testing of 20% SnF_2 in this cement).

Each sample was separately incubated at 37° in 250 ml normal saline. After 24 hrs., the saline was discarded saving only 2 ml of the solution for fluoride analysis. Each flask containing the specimens was again re-filled, incubated, and this process was repeated for 30 days to enable characterization of the leaching of fluoride from each cement. After the 30 day period, the 310 fluoride samples collected were prepared for measurement by diluting them 1/1 with ionic strength buffer (TISAB with CDTA; Orion Res.). The fluoride concentrations were then determined using a fluoride electrode (Orion 90-09 A) connected to a digital readout electrometer (Orion 701) comparing the samples to NaF standards.

In Vivo Tests

Subject

Since 70 percent SnF_2 in polycarboxylate cement demonstrated favorable leaching properties while maintaining compressive strength (see results), in vivo pilot studies with one subject (N.T.) were performed to assess the antiplaque properties of the released fluoride from this cement. After human consent approval, a mesial-occlusal-distal amalgam was removed from a lower right 2nd molar and an orthodontic band was cemented and the tooth restored with the 70 percent SnF_2-polycarboxylate cement.

Two days prior to placement of the temporary restoration, the subject obtained complete plaque removal by means of a toothbrush with the aid of disclosing solution. The subject then abstained from all forms of active oral hygiene for the next 2 days. On day 0 of the experiment (2 days of no oral hygiene), the teeth were stained with disclosing solution (Trace, Lorvic Corp.) and photographs (1:2) of the buccal tooth surfaces were taken. After the temporary restoration was placed, the teeth again were made plaque free and another 2-day no oral hygiene period was begun, terminated by photographs of the plaque and then complete plaque removal. This sequence of 2-day no oral hygiene period and photographs of plaque formation was continued for the 1 month experimental period and post-experimental period of 3 successive months. At the end of the 1 month experimental period, the temporary containing SnF_2 was removed and replaced with polycarboxylate cement without SnF_2.

Plaque Scores

The 4 slides taken on each of the 26 experimental periods were used to determine the extent of visual deposits on the teeth. Plaque scoring was performed according to the method described by Martens & Meskin (1972) using only the buccal surfaces of 20 teeth (from 2nd premolars to 2nd premolars of both maxillary and mandibular arches). The intra-oral slides were examined using a 7x magnifier and a radiographic viewbox. After calibration of 2 examiners (N.T. & ..S.), scoring was performed independently and the mean of the 2 scores was obtained. Both "total deposits" as well as "globular deposits" were recorded. Globular deposits were defined as those deposits that appeared to have thickness and texture. Scores were reduced to mean score per tooth, and a mean score of 5 represents deposits on all surfaces.

Salivary and Urinary Fluoride Levels

To determine salivary and urinary fluoride levels, whole saliva and urine samples were obtained prior to and each day of the 1 month experimental period. Whole salivary samples and urine samples were collected at the same time of each day. Samples were frozen to prevent bacterial growth and warmed to room temperature before fluoride measurements.

SEM and Percent Stannous Fluoride Remaining in Temporary

A fragment of the removed SnF_2-polycarboxylate temporary that was removed after 1 month was prepared for scanning electron microscopy. After coating the specimen with gold-palladium, it was examined with a Hitachi H300 with a H3010 scanning attachment at 20 KV. Following microscopy, the sample was weighed, pulverized and suspended in equal parts of deionized water (50 cc's) and TISAB II with CDTA (50 cc) for 24 hours. The solution was then assayed for fluoride ion concentration and the percent of stannous fluoride remaining after one month was calculated.

RESULTS

In Vitro

Compressive Strength

The control samples of polycarboxylate, zinc phosphate, IRM and zinc oxide eugenol, i.e., those without addition of SnF_2, showed compressive strengths of 23.0 ± 1.3, 14.1 ± 2.7, 5.3 ± 1.5, and 0.7 Klbs/in^2, respectively. The compressive strengths of the cements were decreased linearly with addition of SnF_2 to the powder component of the cement. Yet, poly-carboxylate cement still maintained relatively high compressive strength even with large additions of fluoride (Fig. 1). Zinc phosphate cement appeared to be more detrimentally affected by the SnF_2 than polycarboxylate cement. IRM and zinc oxide eugenol had initial low compressive strengths and the addition of SnF_2 inhibited the setting reaction to the extent that these materials were made unsuitable for further preparation.

In the "post-leaching" compressive strength test, SnF_2-polycarboxylate cement, again, was least affected by additions of SnF_2. For example, 60% SnF_2 in polycarboxylate versus 60% SnF_2 in zinc phosphate cement produced post-leaching compressive strengths of 6.2 vs 0.4 Klbs/in^2, respectively. The one month leaching did, however, affect the strength of the fluoride-polycarboxylate cement. While the unleached 70% SnF_2 in polycarboxylate cement was found to have 10.5 ± 1.5, the post-leached 70% SnF_2 in polycar-boxylate cement samples had a compressive strength of 5.9 Klbs/in^2.

Release of Fluoride from Cement

Release of fluoride from the SnF_2-cement mixtures showed that 70% SnF_2 in polycarboxylate cement had the highest release of fluoride over 30 days with a mean of 3.7 ± 2.8 ppm F/day. The release of fluoride from both polycarboxylate and zinc phosphate cements was rather consistent each day.

In all cases, greater fluoride release was found in polycarboxylate cement versus zinc phosphate concentrations of SnF_2 (Fig. 2). The mean fluoride release from the IRM and zinc oxide eugenol cements was low, ranging from 0.1 to 0.4 ppm F/day.

In Vivo

Anti-Plaque Effects

The baseline scoring, i.e., no oral hygiene for 2 days without SnF_2 temporary in place, showed a "total" plaque score of 3.5 ± 0.08 and a globular plaque score of 2.28 ± 0.58. During the experimental month, the mean total plaque score was 2.9 ± 0.43 and the mean globular plaque score was 0.96 ± 0.25. In the month following the experimental period, total plaque returned to baseline levels; whereas, globular plaque displayed a small "carry over" effect (Table 1, Fig. 3).

Salivary and Urinary Fluoride Levels

The pre-experimental baseline for salivary and urinary fluoride were 0.039 ± 0.015 and 1.6 ± 0.5, respectively. The mean salivary fluoride level, during the experimental month, was increased to 1.86 ± 1.32 ppm F with the greatest elevation in the first 2 weeks. The urinary fluoride levels peaked in the first 2 days and returned to normal daily fluctuation after the first week (Fig. 4). The relationship between the elevation in salivary fluoride level and the reduction in the globular plaque score was nonlinear as evidenced by the weak correlation coefficient ($r = -.3$).

SEM and Percent F in Removed Temporary Restoration

Scanning electron micrographs of the temporary restoration, removed after one month, showed small spaces in the cement in the areas approximate to the orthodontic band. The surface of the restoration, exposed to the oral environment, had an amorphous surface with no visual holes (Fig. 5).

55

Fluoride analysis of a 20 mg sample removed from the tooth after in vivo testing revealed that 8.3 mg SnF_2 remained or 41.5% of the restoration's weight was SnF_2. By subtracting the fluoride remaining in the restoration at the end of the trial from the approximate fluoride initially placed in the restoration, we calculate that no more than 57 mg of fluoride was leached during the month.

Clinical Observations

The marginal adaptation and wear of the 70% SnF_2-polycarboxylate was not substantial during the experimental period. The restoration had color change from pale pink to speckled black to ultimately a uniform grey at the end of the trial (Fig. 6). The only side effect noted was a slight metallic taste on the first day and a brown staining on the dorsum of the tongue adjacent to the temporary restoration. Staining of the dentition was not evident.
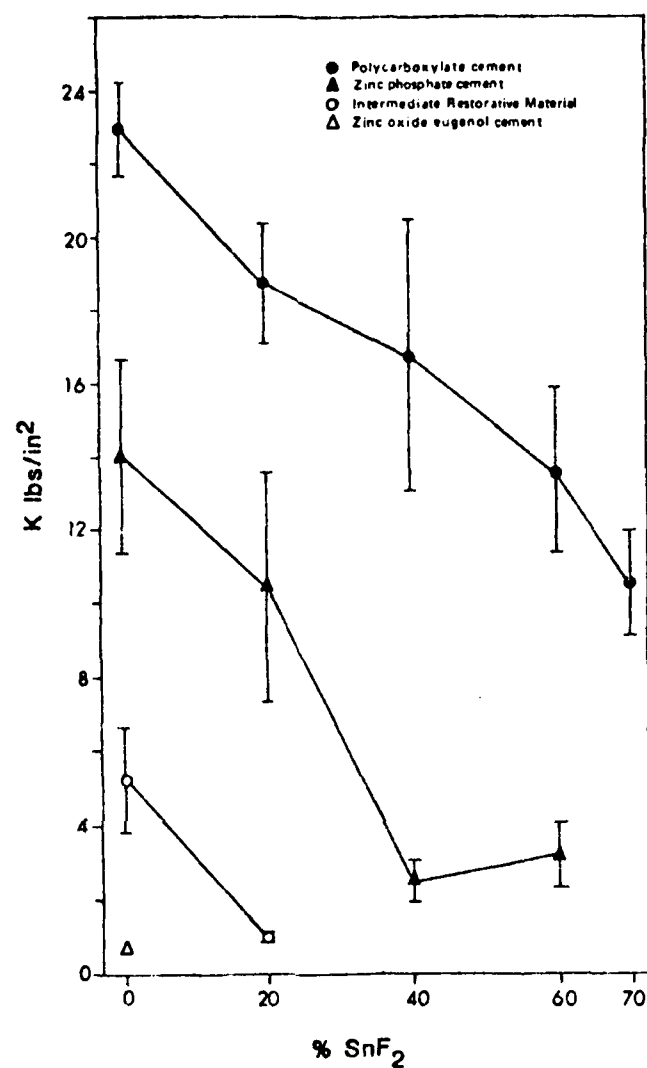
Fig. 1: Ultimate compressive strength (mean ± S.D.) of 4 dental cements containing from 0 to 70% SnF$_2$.
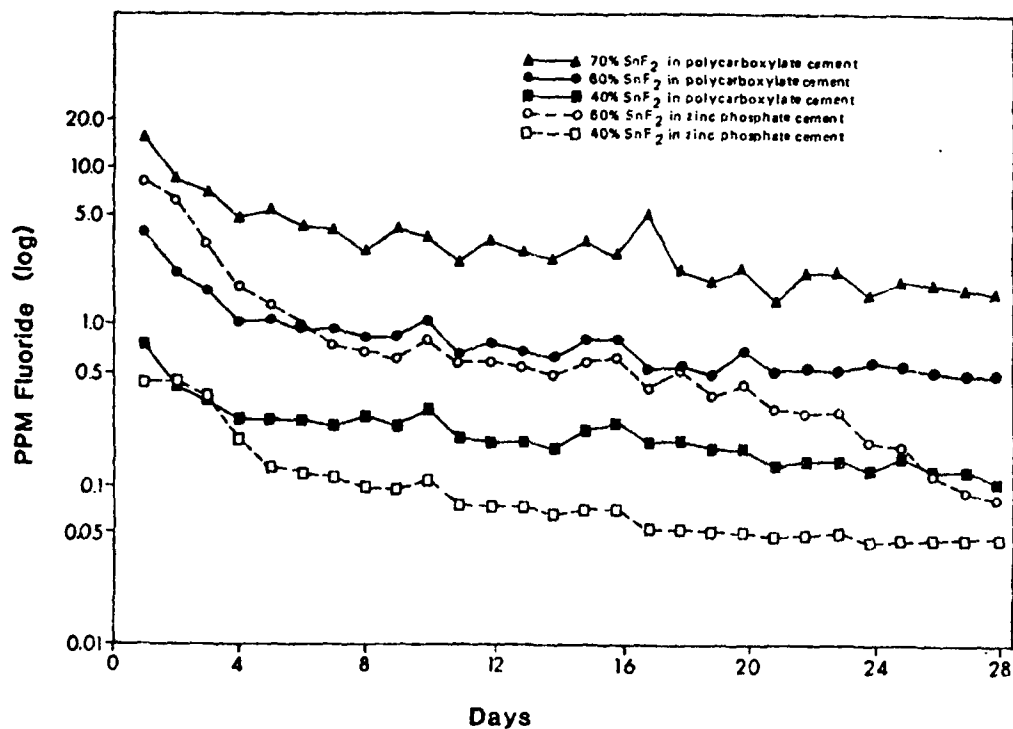
Fig. 2: In vitro release of fluoride from 2 dental cements containing 40 to 70% SnF₂.

58

	Total Plaque	Globular Plaque
Baseline Period	3.5 ± .08	2.28 ± .58
Experimental Period	2.9 ± .43	0.96 ± .25
Month Following Experimental Period	3.5 ± .13	2.08 ± .29

Table 1: Total and globular plaque scores (mean ± S.D.) prior to, during, and after the 30 day experimental period in one subject.
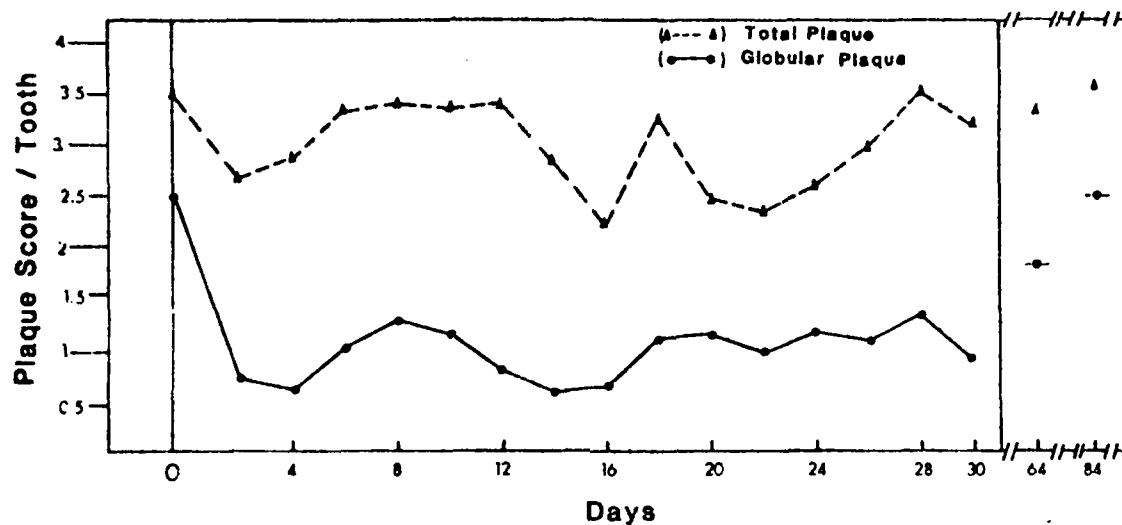
Fig. 3:   Visual plaque (total and globular) scores from subject during
          the 30 day period with the sustained release fluoride restora-
          tion in place and at approximately 1 and 2 months after the
          restorations had been removed.
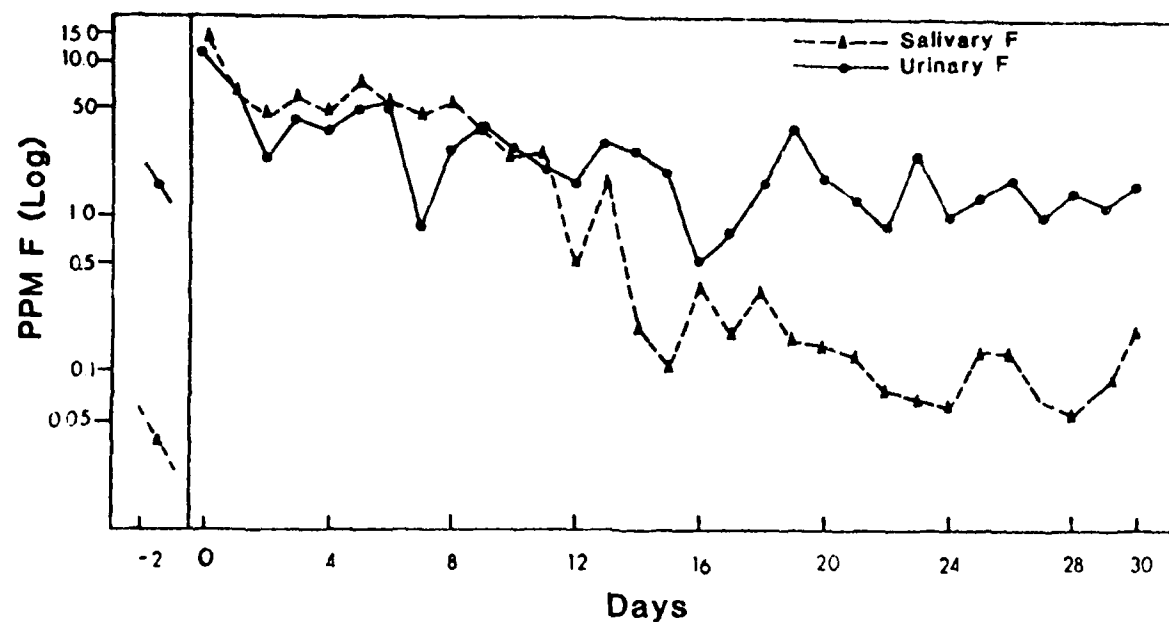
Fig. 4: Daily fluoride concentration in saliva and urine from subject, in the 2 day baseline period and in the 30 day period with the sustained release fluoride restoration in place.
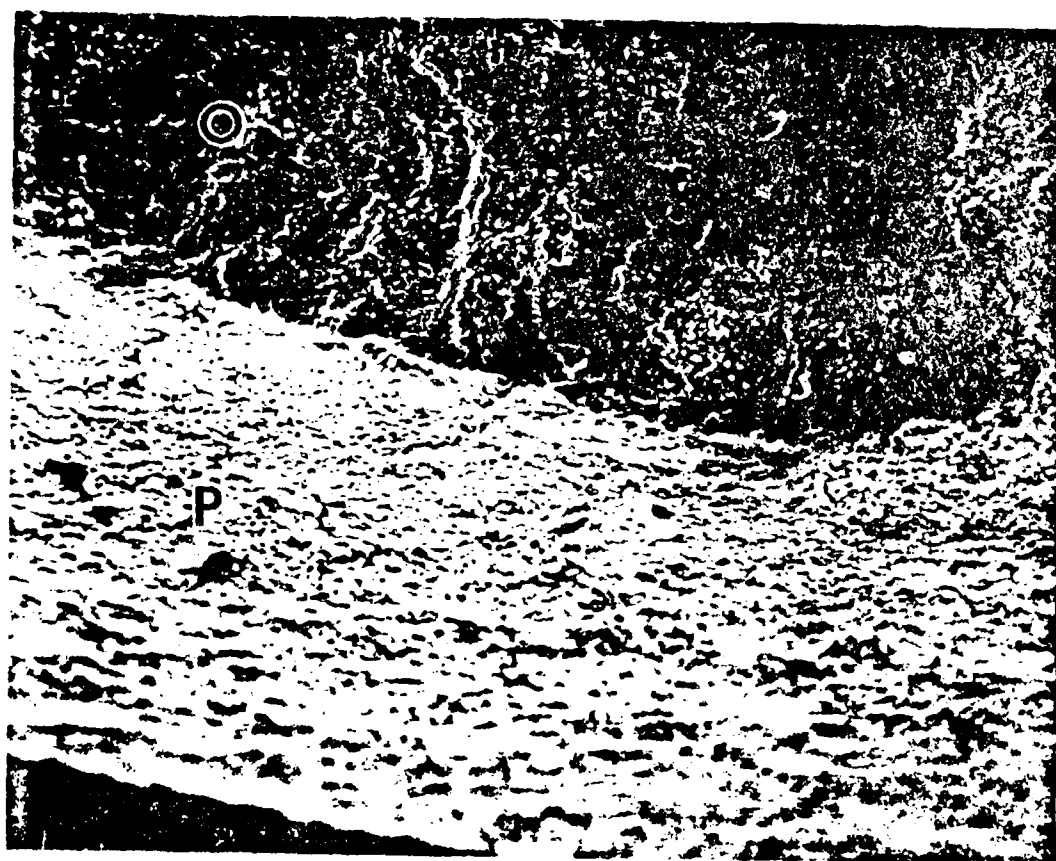
Fig. 5: A low power, 500x, scanning electron micrograph of proximal (P) and occlusal (O) surfaces of SnF_2-polycarboxylate restoration after one month in vivo.
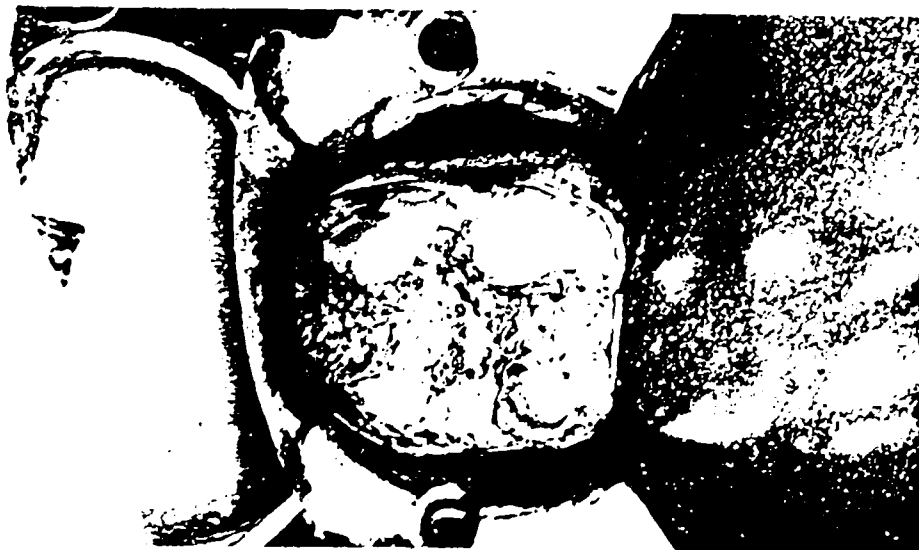
Fig. 6:   Condition of the SnF_2-polycarboxylate temporary restoration at
the end of the 30 day experimental period.

DISCUSSION

These preliminary sustained release experiments designed to evaluate an intracoronal (within the tooth) SnF_2 delivery system both in vitro and in vivo show that besides liberating fluoride for the one month test period, the released fluoride had measurable antiplaque properties in the one test subject.

As shown by the ultimate compressive strength tests, the compatability of large additions of pulverized SnF_2 in polycarboxylate cement was remarkable. Others have reported that additives such as alumina and SnF_2 can actually increase the strength of polycarboxylate cement (Smith, 1978). Even though we found 70% SnF_2 in polycarboxylate cement reduced the compressive strength by about one-half, clinically the material showed sufficient strength in the one month test period. In our mechanical tests, we did not follow exact ADA specifications for testing dental cements (ADA spec. #8 and 61) and consequently, our results vary from others (Phillips et al., 1970; Smith, 1971). However, the different testing procedures would not affect the relative results of one cement tested with various concentrations of fluoride.

The in vitro tests to examine the release pattern of fluoride from the various cements demonstrated that fluoride leached from these materials in a consistent pattern. The release of fluoride was elevated in the first few days for all cements and the release levels were related to the percent SnF_2 in the cements. Due to the favorable release patterns and compressive strength of polycarboxylate cement with 70% SnF_2, we obtained human use approval for in vivo trials in one subject using this cement as a intracoronal restoration.

The 30 day, one subject trial of the 70% SnF_2-polycarboxylate temporary

restorations showed an initial peak release of fluoride followed by a longer

sustained release comparable to that which was similar to the in vitro trial.

In the first day, the fluoride content of the saliva reached 15 ppm F and

the level declined over the one month. The lowest recorded fluoride level

in saliva, 0.1 ppm F on day 28, was still higher than the 0.05 ppm F baseline.

The mean salivary fluoride level for the month of 1.86 ppm F was similar to

the 30 day mean of 1.45 ppm F reported by Mirth et al., 1982, from their

trial with the trilaminate fluoride-release device cemented to the buccal

surface of maxillary molars.

Even though the fluoride levels in saliva were initially high and re-

mained elevated during the experimental month, the urinary fluoride levels

were only notably elevated during the first 2 days. By comparing the

weight and fluoride content of the initially placed restoration to that

removed after 30 days, the total fluoride ingestion was estimated to be

more than 50 mg fluoride. The actual amount was less due to loss at mix-

with mixing and occlusal adjustment. The brief elevation of urinary fluoride

and short-lived fluoride ingestion was judged to be inconsequential insofar

not since the through-put only some of the potential ...

extracellular polysaccharides (Ferretti, Tanzer and Tinanoff, 1981). The
increase in extracellular polysaccharide formation and the clinical observa-
tion of increased pellicle-like deposits in those subjects rinsing with
SnF_2 (Tinanoff and Weeks, 1980) made us discriminate between total plaque
and globular plaque. (We have previously noted by phase contrast microscopy
that deposits on teeth that appear flat and textureless have few bacteria
among amorphous matrix.) The marked reduction in globular plaque in the
experimental period infers that there may be fewer bacteria present in the
deposits on the tooth surfaces due to the presence of SnF_2. Further clinical
trials using bacteria per milligram plaque parameter are necessary to con-
firm the finding of less bacteria on teeth of subjects exposed to sustained
release SnF_2.

The observed "carry over" antiplaque effect after the one month trial
with the SnF_2-polycarboxylate restoration cannot be attributed to the non-
fluoride polycarboxylate cement placed in the tooth after day 30, since
polycarboxylate cements, per se, exhibit no antimicrobial activity
(Schwartzman et al., 1980). A "carry over" effect of SnF_2 has not been
noted when this agent has been used as a mouthrinse (Tinanoff et al., ...),
which suggests that sustained delivery of this agent may have more potent
or long term antimicrobial effects.

Finally, the SnF_2-polycarboxylate restoration had no unfavorable
properties in the one month trial. Aside from the staining of the tongue,
no other systemic side effects were noted. Moreover, the integrity of
wear of the restoration was not significant. The incorporated material
release allowed for good retention which .... The ....
usage of this .... not have an anti............. the
.... teeth that .... for the SnF_2 ....
with a permanent ....

mechanical properties, and putative antiplaque properties of the SnF_2-polycarboxylate temporary restoration, clinical trials using microbiologic as well as clinical parameters are indicated to assess the feasibility of this system as an adjunct in the control of caries and periodontal disease.

Abrahams, L. J., Yonese, M., Higuchi, W., Fox, J. L., Charbeneau, G. T. (1970) In vivo remineralization using a sustained topical fluoride delivery system. Journal of Dental Research, 59, 583.

Addy, M.: (1981) In vitro studies into the use of denture base and soft liner materials as carriers for drugs in the mouth. Journal of Oral Rehabilitation, 8:131.

Ainamo, J. (1971). Control of plaque by chemical agents. Journal of Clinical Periodontology, 4:23.

American Dental Association Specification No. 8 for dental zinc phosphate cement. (1969) Journal of the American Dental Association, 74:1566.

ANSI/ADA Specification No. 61 for zinc polycarboxylate cement. (1980) Journal of the American Dental Association, 101:669.

Douglas, W. H. and Walker, D. M. (1973) Nystatin in denture liners, an alternative treatment for denture stomatitis. British Dental Journal, 135:55.

Duperon, D. F. and Jedrychowski, J. Release and enamel uptake of fluoride from a fluoride-containing polycarboxylate cement. (1980) Journal of Pedodontics 4:287.

Ferretti, G. A., Tanzer, J. M., and Tinanoff, N. (1981) The effect of fluoride and stannous ions on S. mutans. I. Viability, growth, acid, and glucan production. Caries Research. (In press).

Forsten, L. (1976) Fluoride release from a fluoride-containing amalgam and two luting cements. Scandanavian Journal of Dental Research, 84:348.

Friedman, M (1980) Fluoride prolonged release preparations for topical use. Journal of Dental Research, 59:1392.

Hamilton, I. R. (1977) Effects of fluoride on enzymatic regulation of bacterial carbohydrate metabolism. Caries Research, 11 (Suppl. 1):262.

Martens, L. V. and Meskin, L. H. (1972) An innovative technique for assessing oral hygiene. Journal of Dentistry for Children, 39:12.

Mazze, R. I., Calverley, R. K. and Smith, N.T. (1977) Inorganic fluoride toxicity. Anesthesiology, 46:265.

Mellberg, J. R. (1977) Enamel fluoride and its anti-caries effects. Journal of Preventive Dentistry, 4:8.

Mirth, D. B., and Bowen, W. H. (1976) Chemotherapy: antimicrobials and method of delivery. Proceedings - Microbial Aspects of Dental Caries Eds., Stiles, H. M., Loesche, W. J. and O'Brien, T. C., Special Supplement of Microbiology Abstracts, 1:49.

Mirth, D. B., Shern, R. J., Adderly, D. D., Emilson, C. G., Gomez, I. M., Kline, L., Li, S. H., and Bowen, W. H. (1981) Clinical study of an intra-oral device for the controlled release of fluoride. I.A.D.R. Abstract #1063.

Phullips, R. W., Swartz, M. L. and Rhodes, B. (1970) An evaluation of a polycar-boxylate adhesive cement. Journal of the American Dental Association. 81:1353.

Smith, D. C. (1971) A review of zinc polycarboxylate cements. Journal of the Canadian Dental Association. 37:22.

Smith, D. C. (1978) An Outline of Dental Materials and Their Selection. (Ed.. O'Brien, W. J. and Ryge, G.), P. 152, W. B. Saunders, Philadelphia.

Schwartzman, B., Caputo, A. A. and Schein, B. (1980) Antimicrobial action of dental cements. Journal of Prosthetic Dentistry, 43:309.

Taves, D. R., Fry, B. W., Freeman, R. B., and Gillies, A. J. (1970) Toxicity following methoxyflurane anesthesia. II. Fluoride concentrations in nephrotoxicity. Journal of the American Medical Association, 214:91.

Tinanoff, N., Hock, J., Camosci, D., and Hellden, L. (1980) Effect of stannous fluoride mouthrinse on dental plaque formation. Journal of Clinical Periodontology. 7:232.

Tinanoff, N., and Weeks, D. B. (1979) Current status of SnF_2 as an antiplaque agent. Pediatric Dentistry, 1:199.

Thomas, C. J., and Nutt, G. (1978) The in vitro fungicidal properties of visco-gel, alone and combined with nystatin and amphoterocin B. Journal of Oral Rehabilitation, 5:167.

Whitford, G. M., Ekstrand, J., Callan, R. S., and Pearson, D. E. (1980) Subcutaneously implanted alginate as a continuous release source of fluoride. Journal of Dental Research, 59:186.

Yeoman, C. M., Greenspan, J. S., and Harding, S. M. (1978) Recurrent oral ulcera-tion. A double-blind comparison of treatment with betamethasone valerate aerosol and placebo. British Dental Journal, 144:114.

Zity, A., Gedalia, I., and Grajower, R. (1981) Addition of fluoride compounds to acrylic resin plates: bending strength and fluoride release. Journal of Oral Rehabilitation, 8:37.

Authors

Thomas D. Swanson
Department of Pediatric Dentistry
School of Dental Medicine
University of Connecticut Health Center
Farmington, CT 06032, U. S. A.

Norman Tinanoff
Department of Pediatric Dentistry
School of Dental Medicine
University of Connecticut Health Center
Farmginton, CT 06032, U. S. A.

The Effect of Fluoride and Stannous Ions on *Streptococcus mutans*

Viability, Growth, Acid, Glucan Production, and Adherence

G. A. Ferretti, J. M. Tanzer, N. Tinanoff

Departments of Pediatric Dentistry and Oral Diagnosis, School of Dental Medicine, University of Connecticut Health Center, Farmington, Conn., USA

Abstract. The effects of various salts of fluoride and tin were assessed on *Streptococcus mutans* NCTC 10449S viability, growth, acid production, glucan, DNA formation, and tin accumulation. SnF, had more potent bacteriostatic and bactericidal effects than SnCl,. NaF, Na,SnF, or TiF,. SnF,. SnCl,, Na,SnF, and NaF (at 10 ppm F or Cl) reduced the growth yield of *S. mutans*, while acid production by this organism appeared to be reduced only in the fluoride-supplemented media. Bacterial growth in fluoride-supplemented media resulted in greater net amounts of both the water-soluble and alkali-soluble glucans per bacterial mass, with SnF, having the greatest effect, increasing the water-soluble component 10 times and the alkali-soluble component 3 times over the controls. Greater tin uptake was noted by cells exposed to SnF, than by those exposed to SnCl,.

Introduction

In addition to its physicochemical interactions with tooth enamel, fluoride may influence plaque acid production, growth and attachment. Evidence of fluoride inhibition of acid production, even at 1 ppm, is well established (*Bibby and Van Kesteren*, 1940; *Hamilton*, 1977). Higher concentrations of fluoride may affect bacterial growth or viability, and clinical evidence suggests that daily topical application of 1.23% (12.300 ppm) fluoride as NaF (pH 3.0) reduces human plaque scores (*Loesche* et al., 1975).

Stannous fluoride (100–1,000 ppm) applications reduce plaque in experimental animals (*König*, 1959; *Hock and Tinanoff*, 1979) and humans (*Svatun* et al., 1977; *Yankell* et al., 1980; *Tinanoff* et al., 1980). The more pronounced effect of SnF, than NaF on plaque formation may possibly be due to the effect of the former on bacterial attachment (*Tinanoff* et al., 1976) and/or tin accumulation within bacterial cells (*Tinanoff and Camosci*, 1980).

Because most studies of fluoride or stannous ions have been performed at concentrations that could have been bactericidal or

bacteriostatic, it appears valuable to examine their antiplaque properties at low levels (10 ppm) in order to differentiate between possible antiplaque mechanisms.

Materials and Methods

Enamel Specimen Preparation

Enamel sections approximately 180 mm² were cut from smooth surfaces of bovine incisors using a diamond drill with water coolant. A hole was placed in each specimen so that a 0.030 inch diameter stainless steel wire could be used to suspend it in a culture tube. Specimens were cleaned with as surry of pumice to remove organic material, washed with deionized water in an ultrasonic cleaner, and autoclaved. Inlay casting wax (Kerr Products, Emeryville, Conn.) was used to cover the cut inner aspects of specimens leaving only the intact surface enamel exposed. The specimens were disinfected in 70% ethyl alcohol for 15 min and then rinsed in sterile deionized water for 10 min

The surface area of the enamel slabs was estimated by making 1:1 photographic negatives of specimens and placing them over mm² blocked graph paper, the number of mm² blocks contained within the outline of the enamel specimen being approximately equivalent to the exposed enamel surface area of the specimen. This surface area exposed to test agents and bacteria was used for subsequent calculations.

Microorganisms and Agents

Streptococcus mutans NCTC 10449S [Tanzer et al., 1976] was selected as the test organism since this organism attaches to enamel in a similar way in vivo [Tinanoff et al., 1978], causes caries [Tanzer et al., 1976; Tanzer, 1979]; and is representative of the most frequently found S. mutans serotype in human populations [Bratthall, 1972; Arene et al., 1977]. Stock cultures were maintained by monthly transfer in fluid thioglycolate medium (Difco) supplemented with meat extract (20% v v) and excess CaCO₃. For minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) determinations, cultures were adapted to and grown in trypticase soy broth

(TSB; BBL). For all other experiments, stock cultures were adapted to and grown in complex medium [Jordan et al., 1960] supplemented with 5% sucrose and 50 mg/l Na₂CO₃. All experiments were performed at 37 °C under microaerophyllic conditions.

Fresh aqueous solutions of several fluoride compounds were first prepared at 100 ppm with respect to F, i.e., NaF (0.022% w/v, pH 5.3), SnF₂ (0.041%, pH 3.8), Na₂SnF₆ (0.024%, pH 3.5), and TiF₄ (0.016%, pH 2.9), and then added to the complex medium supplemented to produce fluoride concentrations of 10 ppm. SnCl₂ (0.05%, pH 2.9), equimolar with respect to the Sn in SnF₂ (100 ppm F), was similarly prepared and added to the growth medium. As an F-free, Sn-free control, and equal volume of deionized water was added to the medium. The final pH of the supplemented media in all cases was 7.6.

To insure the accuracy of calculated nominal fluoride levels, free fluoride was determined by fluoride electrode (Orion 90-09A, Orion Research Laboratories, Cambridge, Mass) immediately after addition of the fluoride agent to the media and after incubation of inoculated or uninoculated media for 24 h at 37 C

MIC/MLC Determination of Test Agents

To determine the concentration of the various fluoride or stannous solutions that could either kill or completely inhibit growth of S. mutans, the minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of these agents [Barry, 1976] were determined. TSB culture tubes containing serial dilutions of the presumptive antimicrobials were inoculated such that there were $5.0 \cdot 10^5$ CFU/ml of strain 10449S. After incubation at 37 C for 16-18 h, they were evaluated for turbidity. Because some test agents precipitated, uninoculated controls were used to establish baseline turbidity due to apparent chemical changes of the test agents in broth. The MIC was defined as the lowest concentration of an agent resulting in turbidity no greater than that of the corresponding uninoculated tube. The MIC was defined as the lowest concentration of agent resulting in failure to recover viable microorganisms from inoculated culture tubes at the end of 16-18 h. Viability was tested by plating cultures on blood and Mitis salivarius agars.

Bacterial Growth and Acid Production

To assess growth, the optical density of complex medium cultures containing the various agents at 10 ppm F (or 10 ppm Cl in the case of SnCl₂) was monitored at hourly intervals after inoculation by cultures adapted to the same medium without the test agents. Optical density was measured with a Spectronic 20 Spectrophotometer (Bausch & Lomb, Rochester, N.Y.) at 600 nm. Simultaneously, the pH of the cultures was measured.

DNA/Glucan Analysis

NaF-, SnF₂-, Na₂SnF₆-, and TiF₄-supplemented media were placed into culture tubes containing the wire-suspended enamel cylinders and were inoculated with 0.1 ml of an *S. mutans* culture adapted to the same medium without the test agents. The enamel specimens were transferred serially every 24 h to fresh media. After 3 days' growth, the wax was removed from each enamel slab leaving bacteria attached only to the surface enamel. The enamel specimens were then sonified (Bronson Model W 185, Heat Systems Ultrasonics, Plainview, N.Y.) with a microprobe tip in deionized water for 30 s at 50 W with the output at 4, directing the probe tip such as to remove all bacterial deposits from the enamel surface, as judged microscopically. The dislodged bacteria were centrifuged (9,000 g, 10 min, 0C) and resuspended in deionized water three times. A sample of the suspended cells and of the spent culture liquor of the third day's incubation was retained for glucan analysis according to the procedure of *Freedman and Tanzer* [1974]. The remainder of the previously adherent cells and the spent culture fluid of the third day's incubation was analyzed for DNA after hot perchloric acid extraction [*Ogur and Rosen*, 1950; *Burton*, 1956, *Tanzer* et al., 1969].

Atomic Absorption Spectrophotometry

After 3 days' growth, the bacteria on wires of each treatment group were pooled into a preweighed glass centrifuge tube, pelleted by centrifugation, and the supernatant fluid removed. Samples were dried for 3 days at 70 C and the tubes re-weighed. After the dry weights of the harvested cells were thus calculated, the samples were suspended in 3.6 M HCl. Tin in the samples and in

standards (SnCl₂, Alfa Chemical, Danvers, Mass.) was measured in triplicate using a Model 403 atomic absorption spectrophotometer (Perkin-Elmer, Stamford, Conn.) equipped with an AGA-74 graphite furnace.

Results

Fluoride Levels in Growth Media

The NaF-, Na₂SnF₆-, and SnF₂-supplemented media exhibited, by fluoride electrode, 10 ppm fluoride immediately after preparation, consistent with their nominal concentrations computed at the weighing of the compounds. After 24 h incubation, however, all three showed a decrease of approximately 1 ppm F in both inoculated and uninoculated media, possibly due to organic binding of fluoride to constituents of the growth medium. Only TiF₄ did not have measured fluoride concentrations equal to their nominal levels; nominal 10 ppm solutions had measured levels of only 2.3 ppm F both in fresh medium and after 24 h incubation of inoculated or uninoculated media.

MIC/MLC

SnF₂ had the lowest MIC and MLC of the fluoride compounds, 60 and 125 ppm F, respectively, when compared according to fluoride ion concentration (table 1). TiF₄, unlike the other agents, had variable MIC and MLC. The MIC for NaF was 300 ppm and its MLC was 10-fold higher. SnCl₂ had a MIC of 200 ppm Cl and MLC of 225 ppm Cl. With respect to tin concentration, SnF₂ had the lowest MIC and MLC, being about 3- and 2-fold more potent in MIC and MLC, respectively, than the other Sn-containing compounds.

Bacterial Acid Production and Growth at Low Fluoride Levels

There were slight effects of the various F- agents or $SnCl_2$ at 10 ppm on the rate of culture pH fall and generation time (fig. 1, 2). Slowing of the generation time was most notable in the presence of SnF_2, $SnCl_2$, and Na_2SnF_6, and differences in growth rate from the fluoride-free and tin-free control could not be observed for TiF_4 and NaF. However, the growth yield in the presence of all of the compounds, except TiF_4, was clearly lower than in their absence. Similarly, NaF, SnF_2, and Na_2SnF_6 slightly retarded the rate of culture pH fall but $SnCl_2$, as well as TiF_4 had no appreciable effect. The terminal pH was not as low for cultures incubated with SnF_2, NaF, or Na_2SnF_6 as for those with $SnCl_2$, TiF_4 or without additive.

DNA and Glucan Analyses

Table II presents the ranking of treatment effects for various test agents and water controls with respect to the amount of DNA and alkali soluble glucan (ASG) per unit enamel surface area, as well as the amount of ASG per DNA.

Less enamel-adherent DNA and alkali soluble glucan (ASG) were found in the presence of SnF_2, Na_2SnF_6, and NaF compared to other compounds tested, with SnF_2 showing the least. However, there was no significant difference in the ratio μg ASG/μg DNA among these samples This suggests that the lower ASG found in the fluoride test groups was due to the presence of fewer bacteria on the enamel in these groups and that these agents, especially SnF_2, interfered with growth or adhesion of bacteria to the enamel No water-soluble glucan was detected in the enamel-adherent cell mass

Comparison of 'total DNA' at the end of 3 days' growth, i.e., enamel-adherent bacterial DNA and culture liquor DNA from the third day's culture fluid, revealed less DNA/ml medium in the presence of the various fluoride salts and $SnCl_2$ than in their absence (table III). The SnF_4-supplemented cultures had the least DNA. However, there were statistically higher ratios of total ASG/DNA (table III) for SnF_2, Na_2SnF_6, and NaF than for TiF_4, $SnCl_2$ and the water control groups. ASG derived from adherent and nonadherent organisms, expressed per milliliter of culture medium increased in the presence of these agents but not in the presence of $SnCl_2$, TiF_4 and water controls. Thus, SnF_2, Na_2SnF_6 and NaF fostered apparent glucan synthesis while inhibiting bacterial growth. The most potent agent in this regard was SnF_2, as shown by the ratios of ASG DNA and WSG/DNA.

Tin Content of Bacteria Adherent to Stainless Steel Wire

As expected, no tin was detected in the 3-day, wire-adherent bacteria in the control, TiF_4, and NaF treatment groups, while the bacteria grown in the presence of SnF_2, $SnCl_2$, and Na_2SnF_6 contained tin. The plaque incubated in SnF_2-supplemented media had more tin mg plaque than in those specimens cultured in $SnCl_2$- or Na_2SnF_6-supplemented media (table IV).

Discussion

The relatively high bacteriostatic and bactericidal activity observed for SnF_2 at low concentrations cannot be explained by the separate action of stannous or fluoride

ions alone, since neither $SnCl_2$ nor NaF had MIC or MLC values nearly as low as SnF_2. The greater potency of SnF_2 than NaF and $SnCl_2$ has been previously observed [*Tinanoff* et al., 1976; *Tinanoff and Camosci*, 1980]. The mechanism(s) for these differences is suggested by other findings in this study.

Although several experiments showed an effect of stannous ions on *S. mutans*, the alteration of acid production seems to be due primarily to fluoride because $SnCl_2$ at the levels tested had no detectable effect on the rate of culture pH fall. Inhibition of acid production by salivary and plaque bacteria by less than 1 ppm F has been known for some time [*Bibby and Van Kesteren*, 1940; *Wright and Jenkins*, 1954]. Furthermore, plaque collected from subjects living in fluoridated areas exhibits less acid production on exposure to sucrose than plaque from subjects living in nonfluoridated areas [*Jenkins and Edgar*, 1969]. These findings may be at least partially explained by the observation that fluoride interferes with enolase, essential for glycolysis and the energetics supporting membrane transport of glucose and sucrose [*Hamilton*, 1977, *Slee and Tanzer*, 1979].

While insoluble cell-associated glucan synthesis probably contributes to the optical density of cultures of *S. mutans* grown in the presence of sucrose, it is well established that there is a high correlation between optical density and culture DNA [*Tanzer* et al., 1969, 1973; *Robrish* et al., 1971] It is thus notable that bacterial growth yields were lower in all media supplemented with either fluoride or tin compounds except for TiF_4 (recall that the level of TiF_4 tested was lower than 10 ppm) The decreased growth rate and yield may be due in part to the altered

carbohydrate metabolism of *S. mutans* in the presence of fluoride, as is suggested by the increased apparent total culture glucan in its presence. Furthermore, heavy metals, such as tin, are known to have a 'germicidal' effect because of their ability to precipitate proteins [*Salle*, 1968]. Therefore, tin may be metabolically disruptive, accounting for the detectably decreased growth in its presence, compared with the growth in the presence of NaF at the same low concentration.

There was a decrease in bacterial DNA and glucan attached to enamel specimens exposed to NaF, SnF_2 or Na_2SnF_6, with SnF_2 having the greatest effect. No differences were noted in the amount of enamel-adherent ASG among groups when these data were normalized for the variations in bacterial quantity. Consequently, the decreased enamel-adherent alkali soluble glucans (ASG/mm^2) in media supplemented with fluorides may be explained as resulting from either reduction of bacterial adherence to the enamel or reduction in bacterial growth.

Although no differences in enamel-attached glucans due to fluoride or tin were found, an overall increase in 'total' water and alkali-soluble glucan for those test groups exposed to NaF, SnF_2 and Na_2SnF_6 was observed. The total glucan calculation represents the enamel attached and unattached cell-associated glucans (alkali soluble glucan) and WSG component in the media. This increase in both alkali and water soluble glucan components was most evident in the SnF_2 treatment groups with 4 times more alkali soluble and 10 times more water soluble glucan being produced in the SnF_2 group as compared to the control

A problem in this experimental design is

the calculation of total DNA because bacteria adherent to the wire were removed for tin analysis. Yet, since the bacterial dry weight used for tin analysis was similar for each group, there was no significant effect on the glucan DNA ratios computed.

Most studies that have evaluated the effect of fluoride on bacterial extracellular polysaccharide (EPS) production have reported decreases under the influence of fluoride concentrations ranging from 10 to 70 ppm F [*Loesche* et al., 1973, 1975, *Bowen and Hewitt*, 1974]. Recently, *Treasure and Handelman* [1980] verbally reported extracellular polysaccharide synthesis bacterial protein data for several strains of *S. mutans* incubated under the influence of 25 or 50 ppm F. In contrast to the earlier studies, they found increased amounts under the influence of fluoride, consistent with the present data. Whether the increased extracellular glucan yields found in the presence of fluoride results from increased synthesis of glucans, decreased dextranase activity and or channeling of carbon flow away from the partially inhibited glycolytic pathway toward extracellular polysaccharide synthesis needs further study.

We found greater tin uptake in SnF_2 treated cells than $SnCl_2$-treated ones. *Rølla* [1976] and *Svatun* et al. [1977] have suggested that tin ions may compete with calcium for acidic groups on the bacterial surface, thus concentrating this cation on the cell surface. However, increased tin in bacterial cells exposed to SnF_2 could possibly result directly or indirectly from accumulation of fluoride by bacteria. *Jenkins and Edgar* [1980] fluoride is accumulated by plaque [*Jenkins and Edgar*, 1978], whereas fluoride apparently is not concentrated by bacteria [*Mitchell and Maxson*, 1965, *Schule*,

et al., 1962] (It should be noted that $SnCl_2$ and SnF_2 solutions were formulated for equimolar Sn concentrations. SnF_2 and Na_2SnF_4 were adjusted for equimolar F concentrations, not Sn concentrations. This may account for the lower tin uptake from Na_2SnF_4 compared to SnF_2.)

SnF_2 appears to have the most significant antiplaque properties against *S. mutans* of those fluoride compounds tested at a concentration of 10 ppm F. The increased effectiveness of SnF_2 appears related to increased cellular tin accumulation.

References

Bazin, A.T. The settings for susceptibility tests. Principles and practices (ed. A. Lehigh). Philadelphia 1976.

Hibbs, R.G. Van Reswen, M. The effect of fluoride on oral bacteria. J. den. Res. 19: 301-302, 1940.

Bowen, W.H. Hewitt, J.F. Effect of fluoride on extracellular polysaccharide production by Streptococcus mutans. J. den. Res.

Handelman, D. Treasure, E.

Hamilton, R.

Rølla,

Svatun, M.

Jenkins,

Mitchell,

Schule,

lism Caries Res. *11* suppl. 1, pp. 262–291 (1977).

Hock, J., Tinanoff, N.: Resolution of gingivitis following topical application of 0.4% stannous fluoride and toothbrushing. J. dent. Res. *58*: Ir. 2–1653 (1979).

Jenkins, G.N., Edgar, W.M.: The distribution and metabolic effects of human plaque fluorine. Archs oral Biol. *14*: 105–119 (1969).

Jordan, H.R., Fitzgerald, R.J., Bowler, A.: Inhibition of experimental caries by sodium metabisulfite and its effect on the growth and metabolism of selected bacteria. J. dent. Res. *39*: 116–123 (1960).

Keene, H., Shklair, I., Mickel, G.: Effect of multiple dental floss-SnF₂ treatment on *Streptococcus mutans* in interproximal plaque. J. dent. Res. *56*: 21–27 (1977).

Konig, K.G.: Dental caries and plaque accumulation in rats treated with stannous fluoride and penicillin. Helv. odont. Acta *3*: 39–44 (1959).

Loesche, W.J.: Topical fluorides as an antibacterial agent. J. prev. Dent. *4*: 21–25 (1977).

Loesche, W.J., Murray, R.J., Mellberg, J.: The effect of topical acidulated fluoride on percentage of *Streptococcus mutans* and *Streptococcus sanguis* in interproximal plaque samples. Caries Res. *7*: 283–296 (1973).

Loesche, W.J., Syed, S.A., Murray, R.J., Mellberg, J.R.: Effect of topical acidulated phosphate fluoride on percentage of *Streptococcus mutans* and *Streptococcus sanguis* in plaque. Caries Res. *9*: 139–155 (1975).

Mitchell, P., Moyle, J.: Permeability of envelopes of *Staphylococcus aureus* to some salts, amino acids and non-electrolytes. J. gen. Microbiol. *19*: 434–441 (1959).

Ogur, M., Rosen, G.: The nucleic acids of plant tissues. I. The extraction and estimation of desoxypentose nucleic acid and pentose nucleic acid. Archs Biochem. *25*: 261–276 (1950).

Robrish, S.A., LeRoy, A.F., Chassey, B.M., Wilson, J.J., Krichevsky, M.I.: Use of a fiber optic probe for spectral measurements and the continuous recording of the turbidity of growing microbial cultures. Appl. Microbiol. *21*: 278 (1971).

Rolla, G.: The effects of fluoride on initiation of plaque formation. Caries Res. *11* suppl. 1, pp. 243–261 (1976).

Salle, A.J.: Heavy metals other than mercury and silver; in Laurence, Block, Disinfection, sterilization and preservation, pp. 321–327 (Lea & Febiger, Pennsylvania 1968).

Schultz, S.F.; Wilson, W.C.; Epstein, W.: Cation transport in *Escherichia coli*. II. Intracellular chloride concentrations. J. gen. Physiol. *46*: 159–165 (1962).

Shern, R.J.; Couet, K.M.: Effects of SnF₂ and DAPA-1 on existing dental plaque rats. AADR Abstr. No. 637 (1977).

Slee, A.M.; Tanzer, J.M.: Phosphoenolpyruvate-dependent sucrose phosphotransferase activity in *Streptococcus mutans* NCTC 10449S. Infect. Immunity *24*: 821–838 (1979).

Svatun, B.; Gjermo, P.; Eriksen, H.M.; Rölla, G.: A comparison of plaque-inhibiting effect of stannous fluoride and chlorhexidine. Acta odont. scand. *35*: 247–256 (1977).

Tanzer, J.M.: Essential dependence of smooth surface caries on and augmentation of fissure caries by sucrose and *Streptococcus mutans* infection. Infect. Immunity *25*: 526–531 (1979).

Tanzer, J.M.; Brown, A.T.; McInerney, M.F.: Identification, preliminary characterization and evidence for regulation of invertase in *Streptococcus mutans*. J. Bact. *116*: 192–202 (1973).

Tanzer, J.M.; Freedman, M.L.; Woodiel, F.N.; Eifert, R.L.; Rinehimer, L.A.: Association of *Streptococcus mutans* virulence with synthesis of intracellular polysaccharide. Proceedings. Microbial aspects of dental caries. Sp. Supp. microbiol. Abstr. *3*: 597–616 (1976).

Tanzer, J.M.; Wood, W.I.; Krichevsky, M.I.: Linear growth kinetics of plaque-forming streptococci in the presence of sucrose. J. gen. Microbiol. *58*: 125–133 (1969).

Tinanoff, N.; Brady, J.M.; Gross, A.: The effect of NaF and SnF₂ mouthrinses on bacterial colonization of tooth enamel. TEM and SEM studies. Caries Res. *10*: 415–426 (1976).

Tinanoff, N.; Camosci, D.A.: Microbiological, ultrastructural and spectroscopic analysis of the anti-tooth-plaque properties of fluoride compounds in vitro. Archs oral Biol. *25*: 531–543 (1980).

Tinanoff, N.; Hock, J.; Camosci, D.; Hellden, L.: The effect of stannous fluoride mouthrinse on dental plaque. J. clin. Periodont. *7*: 232–241 (1980).

Tinanoff, N.; Tanzer, J.M.; Freedman, M.L.: In
    vitro colonization of *Streptococcus mutans* on
    enamel. Infect. Immunity *21:* 1010–1019
    (1978).
Treasure, P.; Handelman, S.L.: Production of ex-
    tracellular polysaccharides by oral bacteria.
    Trace element interactions. AADR Abstr.
    No. 741 (1980).
Wright, E.E.: Jenkins, G.N.: The effect of fluoride
    on the acid production of saliva glucose mix-
    tures. Br. dent. J. *96:* 30–33 (1954).
Yankell, S.L.; Paskow, G.W.; Shern, R.J.: Effects
    of SnF$_2$ mouthrinses on plaque microbiology.
    IADR Abstr. No. 1109 (1978).

Norman Tinanoff, DDS, MS,
Department of Pediatric Dentistry,
School of Dental Medicine,
University of Connecticut Health Center,
Farmington, CT 06032 (USA)

Fig. 1. Acid production by *S. mutans* NCTC
10449S in medium supplemented with 5% sucrose
and various fluoride compounds (10 ppm F) or
SnCl$_2$ (10 ppm Cl).

Fig. 2. Growth of *S. mutans* NCTC 10449S in
medium supplemented with 5% sucrose and var-
ious fluoride compounds (10 ppm F) or SnCl$_2$
(10 ppm Cl).

1

Caries Ferretti 646

Table I. Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of various fluoride compounds and SnCl, against S. mutans NCTC 10449S

Test compound	MIC		MLC	
	ppm F	ppm Sn	ppm F	ppm Sn
SnF₂	60	180	125	375
SnCl₂	(200 ppm Cl)	600	(225 ppm Cl)	675
Na₂SnF₆	600	600	675	675
NaF	300		3,000	
TiF₄	550±25		575±25	

Caries Ferretti 646

Table II. Amount of bacterial DNA and alkali-soluble glucan (ASG) adherent to enamel after 3 days' incubation of S. mutans NCTC 10449S in medium supplemented with various fluoride compounds (10 ppm F), SnCl₂ (10 ppm Cl) or H₂O (control)

µg DNA/mm² enamel		Subsets	µg ASG/mm² enamel		Subsets	µg ASG/µg DNA		Subsets
Control	0.17±0.02	│	TiF₄	1.35±0.43	│	TiF₄	9.02±1.24	│
SnCl₂	0.15±0.00	│	SnCl₂	1.19±0.05	│	SnCl₂	7.42±0.85	│
TiF₄	0.14±0.03	│	Control	1.10±0.16	│	NaF	7.18±0.70	│
Na₂SnF₆	0.12±0.02	│	NaF	0.83±0.19	│	SnF₂	6.72±1.28	│
NaF	0.11±0.12	│	Na₂SnF₆	0.62±0.04	│	Control	6.39±0.59	│
SnF₂	0.05±0.00	│	SnF₂	0.32±0.09	│	Na₂SnF₆	5.37±0.95	│

Mean of 3 samples ± SD.
Homogeneous subsets using analysis of variance with Scheffe procedure (p < 0.01).

Table III. Total bacterial DNA, alkali-soluble glucan (ASG), and water soluble glucan (WSG) adherent to enamel and present in the culture fluid of the third day's incubation of S. mutans NCTC 104495 growth medium was supplemented with either fluoride compounds (10 ppm F), SnCl (10 ppm Cl), or H2O (control)

Total μg DNA/ml		Subsets	Total μg ASG/ml		Subsets	Total μg ASG/μg DNA		Subsets
Control	3.56±0.41		Na2SnF6	58.1±12.0		SnF	33.2±3.6	
NaF	2.87±0.86		NaF	55.2±2.2		Na2SnF6	23.3±10.7	
Na2SnF6	2.72±0.75		SnF2	55.1±13.0		NaF	20.2±5.3	
TiF4	2.66±0.57		TiF4	33.0±7.9		TiF4	12.4±0.45	
SnCl2	2.52±0.20		Control	32.9±3.8		Control	9.3±1.3	
SnF2	1.27±0.11		SnCl2	21.7±2.6		SnCl2	8.6±0.81	

Total μg WSG/ml		Subsets	Total μg WSG/μg DNA		Subsets
NaF	57.5±7.8		SnF2	45.0±11.3	
SnF2	56.6±10.5		NaF	21.4±7.2	
Na2SnF6	47.2±8.3		Na2SnF6	11.1±3.9	
TiF4	17.7±4.8		TiF4	6.7±1.6	
Control	15.5±0.9		SnCl2	4.2±1.3	
SnCl2	10.9±4.0		Control	4.0±0.48	

Mean of 3 samples ± SD.
Homogeneous subsets using analysis of variance with Scheffe procedure (p < 0.01).

Carles Ferretti 646

Table IV. Tin content of bacteria harvested from the wires suspending enamel specimens of various F, Sn or control groups

Plaque dry weight, mg	Sn/total sample, ppm	Sn/mg plaque, μg

80

2

Control	1.8	ND	ND
Na₂SnF₆	1.5	6	4
TiF₄	3.0	ND	ND
SnCl₂	2.4	48	20
NaF	1.7	ND	ND
SnF₂	1.4	47	34

Samples were pooled, dried, and analyzed for tin using atomic absorption spectrophotometry. The limit of detection of total SN using this method is <1 ppm. ND – Non detected.
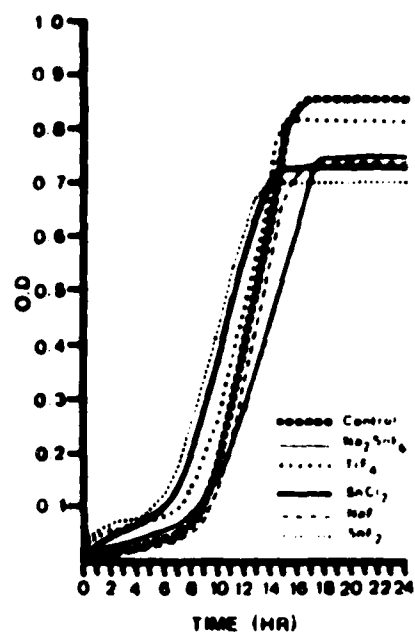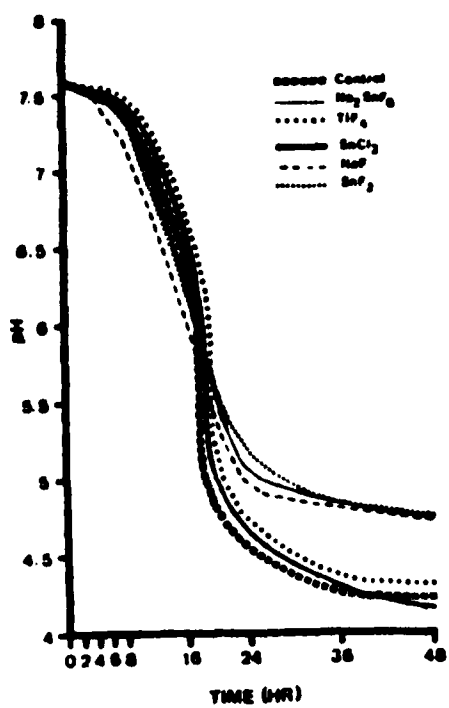
Appendix D

Proposal to the Food and Drug Administration to obtain "Investigational Exemption for a New Drug" to allow for human clinical trials with controlled release SnF_2.

February 4, 1982

Food and Drug Administration
Document Control Section, HFD-106
New Drug Evaluation
bureau of Drugs
5600 Fishers Lane
Rockville, MD 20857

Dear Sir:

Enclosed is a "Notice of Claimed Investigational Exemption for a New Drug"
to use stannous fluoride in a controlled release system. SnF_2 has been
used since the 1950's, and is generally regarded as safe when used as a
treatment to prevent dental caries. Sodium fluoride is used topically,
systematically, and has also been investigated as a controlled release
agent.

Because of the potential antiplaque properties of SnF_2, SnF_2 may be superior
to NaF in a controlled release system. Since this is a new way of delivery
of SnF_2, the U.S. Army Institute of Dental Research, my sponsor, has asked
me to request an "exemption for a new drug" so that I can conduct the enclosed
human clinical trials.

Sincerely,

Norman Tinanoff, D.D.S., M.S.
Associate Professor

NT:jdb

Norman Tinanoff, D.D.S., M.S.
Department of Pediatric Dentistry
School of Dental Medicine
University of Connecticut Health Center
Farmington, CT 06032

MAR 11 1982

Dear Dr. Tinanoff:

Reference is made to your correspondence dated February 4, 1982 which
you intended as a Notice of Claimed Investigational Exemption for a
New Drug (IND) to study stannous fluoride.

We are returning the forms to you per the telephone conversation on
February 17, 1982 between you and Mrs. Joyce. In order to file a complete
IND, each section of the Form 1571 must be answered by furnishing the
information requested or a reference to where the information may be
found. We have the following suggestions to help you address the
inadequacies of your submission:

1.   Submit a signed FD Form 1571 naming yourself as sponsor.

2.   For Parts 2-6 and 16, obtain from Ozark-Mahoning a letter of
     authorization to permit you to incorporate by reference infor-
     mation they may have filed with us.

3.   For Part 7 submit a copy of the labeling that will be used on
     the drug with the statement on the label "For Investigational
     Use Only".

4.   For Parts 11 thru 14, a written statement complying to these
     Parts.

5.   All of this information must be submitted in triplicate in order
     to expedite review by the three review disciplines.

We are enclosing new Forms FD 1571 and 1572 since we had date stamped
the ones submitted.

I hope that this information will be helpful to you. If we can be of
any further assistance, please call Mrs. Regina D. Joyce, Consumer Safety
Officer, at (301) 443-3500.

Sincerely yours,

James P. Mann, M.D.
Director
Division of Surgical-Dental
  Drug Products
Bureau of Drugs

85

Enclosure

THE UNIVERSITY OF CONNECTICUT

HEALTH CENTER

Department of Pediatric Dentistry

March 25, 1982

Food and Drug Administration
Document Control Section, HFD-106
New Drug Evaluation
Bureau of Drugs
5600 Fishers Lane
Rockville, MD 20857

Dear Sir:

Enclosed is a "Notice of Claimed Investigational Exemption for a New Drug" to use stannous fluoride in a controlled release system. SnF_2 has been used since the 1950's, and is generally regarded as safe when used as a treatment to prevent dental caries. Sodium fluoride is used topically, systematically, and has also been investigated as a controlled release agent.

Because of the potential antiplaque properties of SnF_2, SnF_2 may be superior to NaF in a controlled release system. Since this is a new way of delivery of SnF_2, the U.S. Army Institute of Dental Research, my sponsor, has asked me to request an "exemption for a new drug" so that I can conduct the enclosed human clinical trials.

Sincerely,

Tinanoff

Norman Tinanoff, D.D.S., M.S.
Associate Professor
Department of Pediatric Dentistry

NT:1

Enclosure

STATEMENT OF INVESTIGATOR
Clinical Pharmacology

NOTE No drug may be shipped or study initiated unless a completed statement has been received . . . . . .

TO: SUPPLIER OF THE DRUG *Name and address, include ZIP code*

Ozark-Mahoning
1870 South Boulder
Tulsa, Oklahoma 74119

NAME OF INVESTIGATOR . . . . .
Norman Tinanoff, D.D.S., M.S.
DATE
March 25, 1982
NAME OF DRUG
SnF$_2$

Dear Sir
The undersigned, **Norman Tinanoff**
submits this statement as required by section 505(i) of the Federal Food, Drug, and Cosmetic Act and of Title 21 of the Code of Federal Regulations as a condition for receiving and conducting clinical pharmacology with a new drug limited by Federal (or United States) law to investigational use.

1 A STATEMENT OF THE EDUCATION AND TRAINING THAT QUALIFIES ME FOR CLINICAL PHARMACOLOGY

See C.V.

2 THE NAME AND ADDRESS OF THE MEDICAL SCHOOL, HOSPITAL, OR OTHER RESEARCH FACILITY WHERE THE CLINICAL PHARMACOLOGY WILL BE CONDUCTED

Department of Pediatric Dentistry, School of Dental Medicine, University of Connecticut Health Center, Farmington, Connecticut 06032

3 If the experimental project is to be conducted on institutionalized subjects or is conducted by an individual affiliated with an institution which agrees to assume responsibility for the study, assurance must be given that an institutional review committee is responsible for initial and continuing review and approval of the proposed clinical study. The membership must be comprised of sufficient members of varying background, that is, lawyers clergymen, or laymen as well as scientists, to assure complete and adequate review of the research project. The membership must possess not only broad competence to comprehend the nature of the project, but also other competencies necessary to judge the acceptability of the project or activity in terms of institutional regulations, relevant law, standards of professional practice, and community acceptance. Assurance must be presented that the investigator has not participated in the selection of committee members; that the review committee does not allow participation in its review and conclusions by any individual involved in the conduct of the research activity under review (except to provide information to the committee); that the investigator will report to the committee for review any emergent problems, serious adverse reactions, or proposed procedural changes which may affect the status of the investigation and that no such change will be made without committee approval except where necessary to eliminate apparent immediate hazards; that reviews of the study will be conducted by the review committee at intervals appropriate to the degree of risk, but not exceeding 1 year, to assure that the research project is being conducted in compliance with the committee's understanding and recommendations; that the review committee is provided all the information on the research project necessary for its complete review of the project, and that the review committee maintains adequate documentation of its activities and develops adequate procedures for reporting its findings to the institution. The documents maintained by the committee are to include the names and qualifications of committee members, records of information provided to subjects in obtaining informed consent, committee discussion on substantive issues and their resolution, committee recommendations and dated reports of successive reviews as they are performed. Copies of all documents are to be retained for a period of 3 years past the completion or discontinuance of the study and are to be made available upon request to duly authorized representatives of the Food and Drug Administration. (Favorable recommendations by the committee are subject to further appropriate review and rejection by institution officials. Unfavorable recommendations, restrictions, or conditions may not be overruled by the institution officials.) Procedures for the organization and operation of institutional review committees are contained in guidelines issued pursuant to Chapter 1-40 of the Grants Administration Manual of the U.S. Department of Health, Education, and Welfare, available from the U.S. Government Printing Office. It is recommended that these guidelines be followed in establishing institutional review committees and that the committees function according to the procedures described therein. A signing of the Form FD-1572 will be regarded as providing the above necessary assurance; however, if the institution has on file with the Department of Health, Education, and Welfare, Division of Research Grants, National Institutes of Health, an "accepted general assurance," and the same committee is to review the proposed study using the same procedures, this is acceptable in lieu of the above assurances and a statement to this effect should be provided with the signed FD-1572. (In addition to sponsor's continuing responsibility to monitor the study, the Food and Drug Administration will undertake investigations in institutions periodically to determine whether the committees are operating in accord with the assurances given by the sponsor.)

FORM FDA 1572 (8/79)   PREVIOUS EDITION MAY BE USED UNTIL SUPPLY IS EXHAUSTED.

87

See Protocol.

See Protocol.

6 THE UNDERSIGNED UNDERSTANDS THAT THE FOLLOWING CONDITIONS GENERALLY APPLICABLE TO NEW DRUGS FOR INVESTIGATIONAL USE GOVERN HIS RECEIPT AND USE OF THIS INVESTIGATIONAL DRUG

a. The sponsor is required to supply the investigator with full information concerning the preclinical investigation that justifies clinical pharmacology

b. The investigator is required to maintain adequate records of the disposition of all receipts of the drug, including dates, quantity, and use by subjects, and if the clinical pharmacology is suspended, terminated, discontinued, or completed to return to the sponsor any unused supply of the drug. If the investigational drug is subject to the comprehensive Drug Abuse Prevention and Control Act of 1970, adequate precautions must be taken, including storage of the investigational drug in a securely locked, substantially constructed cabinet, or other securely locked, substantially constructed enclosure access to which is limited, to prevent theft or diversion of the substance into illegal channels of distribution.

c. The investigator is required to prepare and maintain adequate case histories designed to record all observations and other data pertinent to the clinical pharmacology.

d. The investigator is required to furnish his reports to the sponsor who is responsible for collecting and evaluating the results, and presenting progress reports to the Food and Drug Administration at appropriate intervals, not exceeding 1 year. Any adverse effect which may reasonably be regarded as caused by, or is probably caused by, the new-drug shall be reported to the sponsor promptly, and if the adverse effect is alarming it shall be reported immediately. An adequate report of the clinical pharmacology should be furnished to the sponsor shortly after completion.

e. The investigator shall maintain the records of disposition of the drug and the case reports described above for a period of 2 years following the date the new-drug application is approved for the drug, or if no application is to be filed or is approved until 2 years after the investigation is discontinued and the

Food and Drug Administration so notified. Upon the request of a scientifically trained and specifically authorized employee of the Department, at reasonable times the investigator will made such records available for inspection and copying. The names of the subjects need not be divulged unless the records of the particular subjects require a more detailed study of the cases, or unless there is reason to believe that the records do not represent actual studies or do not represent actual results obtained.

f. The investigator certifies that the drug will be administered only to subjects under his personal supervision or under the supervision of the following investigators responsible to him.

None

_____

_____

_____

and that the drug will not be supplied to any other investigator or to any clinic for administration to subjects.

g. The investigator certifies that he will inform any patients or any persons used as controls, or their representatives, that drugs are being used for investigational purposes, and will obtain the consent of the subjects, or their representatives, except where this is not feasible or, in the investigator's professional judgment, is contrary to the best interests of the subjects.

h. The investigator is required to assure the sponsor that for investigations involving institutionalized subjects the studies will not be initiated until the institutional review committee has reviewed and approved the study. (The organization and procedure requirements for such a committee should be explained to the investigator by the sponsor as set forth in Form FD-1571, division 10, unit c.)

Very truly yours,

Tinanoff

Name of Investigator

Norman Tinanoff, D.D.S., M.S.
Department of Pediatric Dentistry
Address ~~School of Dental Medicine~~
University of Connecticut Health Center
Farmington, CT 06032

NOTE   No drug may be shipped or study initiated ...

TO: SUPPLIER OF DRUG (Name and address, include zip ...)

NAME ...

Ozark - Mahoning
1870 South Boulder
Tulsa, Oklahoma   74119

Norman Tinanoff

March 25, 1982

SnF₂

Dear Sir:

The undersigned, __Norman Tinanoff__

submits this statement as required by section 505 ... of the Federal Food, Drug, ...

Title 21 of the Code of Federal Regulations as a condition for receiving and ...

with a new drug limited by Federal (or United States) law to investigational use.

STATEMENT OF EDUCATION AND EXPERIENCE

a. COLLEGES, UNIVERSITIES, AND MEDICAL SCHOOLS ... PROFESSIONAL ...
   DEGREES, AND DATES DEGREES WERE AWARDED.

See C. V.

b. POST GRADUATE MEDICAL OR OTHER PROFESSIONAL TRAINING (Indicate dates, name ...

See C. V.

c. TEACHING OR RESEARCH EXPERIENCE (Indicate dates, institutions, and brief description of experience)

See C. V.

d. EXPERIENCE IN MEDICAL PRACTICE OR OTHER PROFESSIONAL EXPERIENCE (Indicate dates, institutional affiliations, nature
   of practice, or other professional experience)

See C. V.

e. REPRESENTATIVE LIST OF PERTINENT MEDICAL OR OTHER SCIENTIFIC PUBLICATIONS (Indicate titles or articles, name of
   publications and volume, page number, and date)

See C. V.

FORM FDA 1573 (4/79)   PREVIOUS EDITION MAY BE USED UNTIL SUPPLY IS EXHAUSTED.

The investigational drug will be used by the undersigned or under his supervision in accordance with the plan of investigation described as follows: (Outline the plan of investigation including approximation of the number of subjects to be treated with the drug and the number to be employed as controls, if any; clinical uses to be investigated; characteristics of subjects by age, sex and condition; the kind of clinical observations and laboratory tests to be undertaken prior to, during, and after administration of the drug; the estimated duration of the investigation; and a description or copies of report forms to be used to maintain an adequate record of the observations and test results obtained. This plan may include reasonable alternates and variations and should be supplemented or amended when any significant change in direction or scope of the investigation is undertaken.)

see Attached Protocol

THE UNDERSIGNED UNDERSTANDS THAT THE FOLLOWING CONDITIONS, GENERALLY APPLICABLE TO NEW DRUGS FOR INVESTIGATIONAL USE, GOVERN HIS RECEIPTS AND USE OF THIS INVESTIGATIONAL DRUG.

a. The sponsor is required to supply the investigator with full information concerning the preclinical investigations that justify clinical trials, together with fully informative material describing any prior investigations and experience and the possible hazards, contraindications, side effects, and precautions to be taken into account in the course of the investigation.

b. The investigator is required to maintain adequate records of the disposition of all receipts of the drug, including dates, quantities, and use by subjects, and if the investigation is terminated, suspended, discontinued, or completed, to return to the sponsor any unused supply of the drug. If the investigational drug is subject to the Comprehensive Drug Abuse Prevention and Control Act of 1970, adequate precautions must be taken including storage of the investigational drug in a securely locked, substantially constructed cabinet, or other securely locked substantially constructed enclosure, access to which is limited, to prevent theft or diversion of the substance into illegal channels of distribution.

c. The investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated with the drug or employed as a control in the investigation.

d. The investigator is required to furnish his reports to the sponsor of the drug who is responsible for collecting and evaluating the results obtained by various investigators. The sponsor is required to present progress reports to the Food and Drug Administration at appropriate intervals not exceeding 1 year. Any adverse effect that may reasonably be regarded as caused by, or probably caused by, the new drug shall be reported to the sponsor promptly, and if the adverse effect is alarming, it shall be reported immediately. An adequate report of the investigation should be furnished to the sponsor shortly after completion of the investigation.

e. The investigator shall maintain the records of disposition of the drug and the case histories described above for a period of 2 years following the date a new-drug application is approved for the drug; or if the application is not approved, until 2 years after the investigation is discontinued. Upon the request of a scientifically trained and properly authorized employee of the Department, at reasonable times, the investigator will make such records available for inspection and copying. The subjects' names need not be divulged unless the records of particular individuals require a more detailed study of the cases, or unless there is reason to believe that the records do not represent actual cases studied, or do not represent actual results obtained.

f. The investigator certifies that the drug will be administered only to subjects under his personal supervision or under the supervision of the following investigators responsible to him,

_____

_____

and that the drug will not be supplied to any other investigator or to any clinic for administration to subjects.

g. The investigator certifies that he will inform any subjects including subjects used as controls, or their representatives, that drugs are being used for investigational purposes, and will obtain the consent of the subjects, or their representatives, except where this is not feasible or, in the investigator's professional judgment, is contrary to the best interests of the subjects.

h. The investigator is required to assure the sponsor that for investigations involving institutionalized subjects, the studies will not be initiated until the institutional review committee has reviewed and approved the study. (The organization and procedure requirements for such a committee should be explained to the investigator by the sponsor as set forth in Form FD 1571, division for

Very truly yours,   *Norman Tinanoff*

Norman Tinanoff, D. D. S., M. D.
Name of Investigator
Department of Pediatric Dentistry
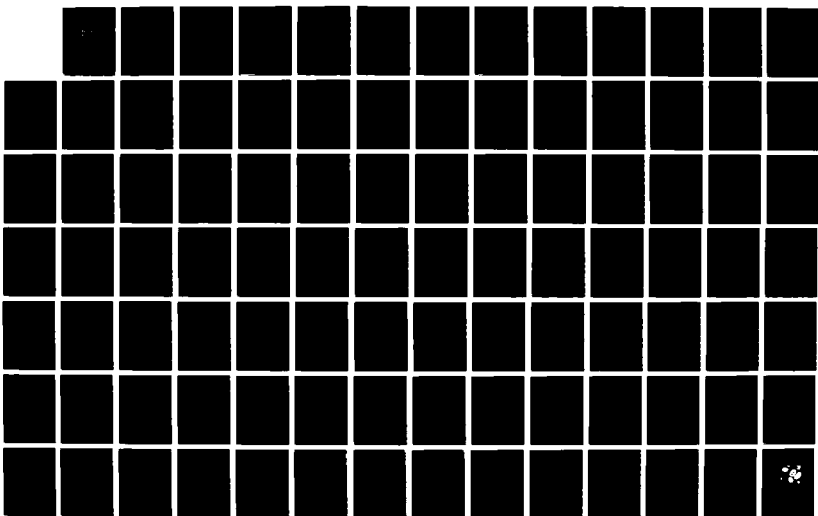University of Connecticut health center

Farmington, CT. 06032
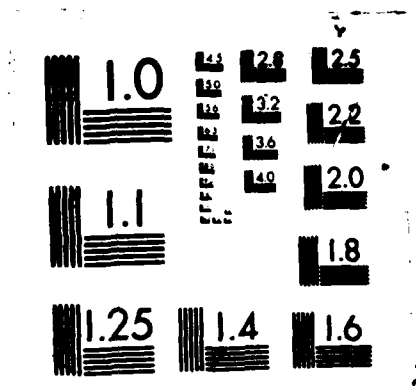
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963-A

NOTICE OF CLAIMED INVESTIGATIONAL EXEMPTION
FOR A NEW DRUG

NOTE: No drug may be shipped or study initiated unless a complete statement has been received (21 CFR 312 1(a)(2))

Name of Sponsor __Norman Tinanoff, D.D.S., M.S.__

Address __Department of Pediatric Dentistry, School of Dental Medicine, University of Connecticut Health Center, Farmington, CT 06032__

Date __March 25, 1982__

Name of Investigational Drug __SnF_2__

To the Secretary of Health, Education and Welfare
For the Commissioner of Food and Drugs
Bureau of Drugs (HFD-106)
5600 Fishers Lane
Rockville, Maryland 20857

Dear Sir:

The sponsor, __Norman Tinanoff__ , submits this notice of claimed investigational exemption for a new drug under the provisions of section 505(i) of the Federal Food, Drug, and Cosmetic Act and §312.1 of Title 21 of the Code of Federal Regulations.

Attached hereto in triplicate are:

1. The best available descriptive name of the drug, including to the extent known the chemical name and structure of any new-drug substance, and a statement of how it is to be administered. (If the drug has only a code name, enough information should be supplied to identify the drug.)

2. Complete list of components of the drug, including any reasonable alternates for inactive components.

3. Complete statement for quantitative composition of drug, including reasonable variations that may be expected during the investigational stage.

4. Description of source and preparation of, any new-drug substances used as components, including the name and address of each supplier or processor other than the sponsor, or each new-drug substance.

5. A statement of the methods, facilities, and controls used for the manufacturing, processing, and packing of the new drug to establish and maintain appropriate standards of identity, strength, quality, and purity as needed for safety and to give significance to clinical investigations made with the drug.

6. A statement covering all information available to the sponsor derived from preclinical investigations and any clinical studies and experience with the drug as follows:

a. Adequate information about the preclinical investigations, including studies made on laboratory animals, on the basis of which the sponsor has concluded that it is reasonably safe to initiate clinical investigations with the drug. Such information should include identification of the person who conducted each investigation, identification and qualifications of the individuals who evaluated the results and concluded that it is reasonably safe to initiate clinical investigations with the drug and a statement of where the investigations were conducted and where the records are available for inspection, and enough details about the investigations to permit scientific review. The preclinical investigations shall not be considered adequate to justify clinical testing unless they give proper attention to the conditions of the proposed clinical testing. When this information, the outline of the plan of clinical pharmacology, or any progress report on the clinical pharmacology, indicates a need for full review of the preclinical data before a clinical trial is undertaken, the Department will notify the sponsor to submit the complete preclinical data and to withhold clinical trials until the review is completed and the sponsor notified. The Food and Drug Administration will be prepared to confer with the sponsor concerning this action.

b. If the drug has been marketed commercially or investigated (e.g. outside the United States), complete information about such distribution or investigation shall be submitted, along with a complete bibliography of any publications about the drug.

c. If the drug is a combination of previously investigated or marketed drugs, an adequate summary of preexisting information from preclinical and clinical investigations and experience with its components, including all reports available to the sponsor suggesting side-effects, contraindications, and ineffectiveness in use of such components. Such summary should include an adequate bibliography of publications about the components and may incorporate by reference any information concerning such components previously submitted by the sponsor to the Food and Drug Administration. Include a statement of the expected pharmacological effects of the combination.

d. If the drug is a radioactive drug, sufficient data must be available from animal studies or previous human studies to allow a reasonable calculation of radiation absorbed dose upon administration to a human being.

7. A total (one in each of the three copies of the notice) of all informational material, including label and labeling, which is to be supplied to each investigator. This shall include an accurate description of the prior investigations and experience and their results pertinent to the safety and possible usefulness of the drug under the conditions of the investigation. It shall not represent that the safety or usefulness of the drug has been established for the purposes to be investigated. It shall describe all relevant hazards, contraindications, side-effects, and precautions suggested by prior investigations and experience with the drug under investigation and related drugs for the information of clinical investigators.

8. The scientific training and experience considered appropriate by the sponsor to qualify the investigators as suitable experts to investigate the safety of the drug, bearing in mind what is known about the pharmacological action of the drug and the phase of the investigational program that is to be undertaken.

FORM FDA 1571 (4/80)   PREVIOUS EDITIONS ARE OBSOLETE

9. The names and a summary of the training and experience of each investigator and of the individual charged with monitoring the progress of the investigation and evaluating the evidence of safety and effectiveness of the drug as it is received from the investigators, together with a statement that the sponsor has obtained from each investigator a completed and signed form, as provided in subparagraph (12) or (13) of this paragraph, and that the investigator is qualified by scientific training and experience as an appropriate expert to undertake the phase of the investigation outlined in section 10 of the "Notice of Claimed Investigational Exemption for a New Drug." (In crucial situations, phase 3 investigators may be added and this form supplemented by rapid communication methods, and the signed form FD-1573 shall be obtained promptly thereafter.)

10. An outline of any phase or phases of the planned investigations and a description of the institutional review committee, as follows:

a. Clinical pharmacology. This is ordinarily divided into two phases: Phase 1 starts when the new drug is first introduced into man—only animal and in vitro data are available—with the purpose of determining human toxicity, metabolism, absorption, elimination, and other pharmacological action, preferred route of administration, and safe dosage range. phase 2 covers the initial trials on a limited number of patients for specific disease control or prophylaxis purposes. A general outline of these phases shall be submitted, identifying the investigator or investigators, the hospitals or research facilities where the clinical pharmacology will be undertaken, any expert committees or panels to be utilized, the maximum number of subjects to be involved, and the estimated duration of these early phases of investigation. Modification of the experimental design on the basis of experience gained need be reported only in the progress reports on these early phases, or in the development of the plan for the clinical trial. phase 3 The first two phases may overlap and, when indicated, may require additional animal data before these phases can be completed or phase 3 can be undertaken. Such animal tests shall be designed to take into account the expected duration of administration of the drug to human beings, the age groups and physical status, as for example, infants, pregnant women, premenopausal women, of those human beings to whom the drug may be administered, unless this has already been done in the original animal studies. If a drug is a radioactive drug, the clinical pharmacology phase must include studies which will obtain sufficient data for dosimetry calculations. These studies should evaluate the excretion, whole body retention, and organ distribution of the radioactive material.

b. Clinical trial. This phase 3 provides the assessment of the drug's safety and effectiveness and optimum dosage schedules in the diagnosis, treatment, or prophylaxis of groups of subjects involving a given disease or condition. A reasonable protocol is developed on the basis of the facts accumulated in the earlier phases, including completed and submitted animal studies. This phase is conducted by separate groups following the same protocol (with reasonable variations and alternatives permitted by the plan) to produce well-controlled clinical data. For this phase, the following data shall be submitted:

i. The names and addresses of the investigators. (Additional investigators may be added.)

ii. The specific nature of the investigations to be conducted, together with information or case report forms to show the scope and detail of the planned clinical observations and the clinical laboratory tests to be made and reported.

iii. The approximate number of subjects (a reasonable range of subjects is permissible and additions may be made), and criteria proposed for subject selection by age, sex, and condition.

iv. The estimated duration of the clinical trial and the intervals, not exceeding 1 year, at which progress reports showing the results of the investigations will be submitted to the Food and Drug Administration.

c. Institutional review committee. If the phases of clinical study as described under 10a and b above are conducted on institutionalized subjects or are conducted by an individual affiliated with an institution which agrees to assume responsibility for the study, assurance must be given that an institutional review committee is responsible for initial and continuing review and approval of the proposed clinical study. The membership must be comprised of sufficient members of varying background, that is, lawyers, clergymen, or laymen as well as scientists, to assure complete and adequate review of the research project. The membership must possess not only broad competence to comprehend the nature of the project, but also other competencies necessary to judge the acceptability of the project or activity in

terms of institutional regulations, relevant law, standards of professional practice, and community acceptance. Assurance must be presented that neither the sponsor nor the investigator has participated in selection of committee members, that the review committee does not allow participation in its review and conclusions by any individual involved in the conduct of the research activity under review (except to provide information to the committee), that the investigator will report to the committee for review any emergent problems, serious adverse reactions, or proposed procedural changes which may affect the status of the investigation and that no such change will be made without committee approval except where necessary to eliminate apparent immediate hazards; the reviews of the study will be conducted by the review committee at intervals appropriate to the degree of risk, but not exceeding 1 year, to assure that the research project is being conducted in compliance with the committee's understanding and recommendations; that the review committee is provided all the information on the research project necessary for its complete review of the project, and that the review committee maintains adequate documentation of its activities and develops adequate procedures for reporting its findings to the institution. The documents maintained by the committee are to include the names and qualifications of committee members, records of information provided to subjects in obtaining informed consent, committee discussion on substantive issues and their resolution, committee recommendations, and dated reports of successive reviews as they are performed. Copies of all documents are to be retained for a period of 3 years past the completion or discontinuance of the study and are to be made available upon request to duly authorized representatives of the Food and Drug Administration (Favorable recommendations by the committee are subject to further appropriate review and rejection by institution officials. Unfavorable recommendations, restrictions, or conditions may not be overruled by the institution officials.) Procedures for the organization and operation of institutional review committees are contained in guidelines issued pursuant to Chapter 1-40 of the Grants Administration Manual of the U.S. Department of Health, Education and Welfare available from the U.S. Government Printing Office. It is recommended that these guidelines be followed in establishing institutional review committees and that the committees function according to the procedures described therein. A signing of the Form FD-1571 will be regarded as providing the above necessary assurances. If the institution, however, has on file with the Department of Health, Education, and Welfare, Division of Research Grants, National Institutes of Health, an "accepted general assurance," and the same committee is to review the proposed study using the same procedures, this is acceptable in lieu of the above assurances and a statement to this effect should be provided with the signed FD-1571. (In addition to sponsor's continuing responsibility to monitor the study, the Food and Drug Administration will undertake investigations in institutions periodically to determine whether the committees are operating the accord with the assurances given by the sponsor.)

(The notice of claimed investigational exemption may be limited to any one or more phases, provided the outline of the additional phase or phases is submitted before such additional phases begin. This does not preclude continuing a subject on the drug from phase 2 to phase 3 without interruption while the plan for phase 3 is being developed.)

Ordinarily, a plan for clinical trial will not be regarded as reasonable unless, among other things, it provides for more than one independent competent investigator to maintain adequate case histories of an adequate number of subjects, designed to record observations and permit evaluation of any and all discernible effects attributable to the drug in each individual treated, and comparable records on any individuals employed as controls. These records shall be individual records for each subject maintained to include adequate information pertaining to each, including age, sex, conditions treated, dosage, frequency of administration of the drug, results of all relevant clinical observations and laboratory examinations made, adequate information concerning any other treatment given and a full statement of any adverse effects and useful results observed, together with an opinion as to whether such effects or results are attributable to the drug under investigation.

11. A statement that the sponsor will notify the Food and Drug Administration if the investigation is discontinued, and the reason therefor.

12. A statement that the sponsor will notify each investigator if a new drug application is approved, or if the investigation is discontinued.

13. If the drug is to be sold, a full explanation why sale is required and

2

93

should not be regarded as the commercialization of a new drug for which an application is not approved.

14. A statement that the sponsor assures that clinical studies in humans will not be initiated prior to 30 days after the date of receipt of the notice by the Food and Drug Administration and that he will continue to withhold or to restrict clinical studies if requested to do so by the Food and Drug Administration prior to the expiration of such 30 days. If such request is made, the sponsor will be provided specific information as to the deficiencies and will be afforded a conference on request. The 30-day delay may be waived by the Food and Drug Administration upon a showing of good reason for such waiver; and for investigations subject to institutional review

committee approval as described in item 10c above, and additional statement assuring that the investigation will not be initiated prior to approval of the study by such committee.

15. When requested by the agency, an environmental impact analysis report pursuant to §25.1 of this chapter

16. A statement that all nonclinical laboratory studies have been, or will be, conducted in compliance with the good laboratory practice regulations set forth in Part 58 of this chapter, or, if such studies have not been conducted in compliance with such regulations, a statement that describes in detail all differences between the practices used in conducting the study and those required in the regulations

Very truly yours,

SPONSOR	PER
Norman Tinanoff, D.D.S., M.S.	Norman Tinanoff, D.D.S., M.S.
	INDICATE AUTHORITY
	Principal Investigator

(This notice may be amended or supplemented from time to time on the basis of the experience gained with the new drug. Progress reports may be used to update the notice.)

ALL NOTICES AND CORRESPONDENCE SHOULD BE SUBMITTED IN TRIPLICATE.

THE UNIVERSITY OF CONNECTICUT
HEALTH CENTER

March 30, 1982

Director of Research
Ozark-Mahoning
1870 South Boulder
Tulsa, OK 74116

Dear Sir:

I am submitting a protocol to the Food and Drug Administration to apply for an "investigational exemption for a new drug". Specifically, my protocol entails incorporating stannous fluoride into a dental cement which is then used as a temporary restoration for the purpose of slowly releasing the stannous fluoride supplied by your company, the F.D.A. has asked me to write to you to obtain a letter of authorization to permit incorporation of any reference information that you may have concerning this substance.

Their request, from F.D.A. Form 1571, specifically states:

2. Complete list of components of the drug, including any reasonable alternates for inactive components.

3. Complete statemen tof quantitative composition of drug, including reasonable variations that may be expected during the investigational stage.

4. Description of source and preparation of, any new-drug substances used as components, including the name and address of each supplier or processor, other than the sponsor, or each new-drug substance.

5. A statement of the methods, facilities, and controls used for the manufacturing, processing, and packing of the new drug to establish and maintain appropriate standards of identity, strength, quality, and purity as needed for safety and to give significance to clinical investigations made with the drug.

6. A statement covering all information available to the sponsor derived from preclinical investigations and any clinical studies and experience with the drug as follows:

a Adequate information about the preclinical investigations, including studies made on laboratory animals, on the basis of which the sponsor has concluded that it is reasonably safe to initiate clinical investigations with the drug: Such information should include identification of the person who conducted each investigation; identification and qualifications of the individuals who evaluated the results and concluded that it is reasonably safe to initiate clinical investigations with the drug and a statement of where the investigations were conducted and where the records are available for inspection; and enough details about the investigations to permit scientific review. The preclinical investigations shall not be considered adequate to justify clinical testing unless they give proper attention to the conditions of the proposed clinical testing. When this information, the outline of the plan of clinical pharmacology, or any progress report on the clinical pharmacology, indicates a need for full review of the preclinical data before a clinical trial is undertaken, the Department will notify the sponsor to submit the complete preclinical data and to withhold clinical trials until the review is completed and the sponsor notified. The Food and Drug Administration will be prepared to confer with the sponsor concerning this action.

b. If the drug has been marketed commercially or investigated (e g outside the United States), complete information about such distribution or investigation shall be submitted, along with a complete bibliography of any publications about the drug.

SCHOOL OF DENTAL MEDICINE
FARMINGTON, CONNECTIC

95

c. If the drug is a combination of previously investigated or marketed drugs, an adequate summary of preexisting information from preclinical and clical investigations and experience with its components, including all reports available to the sponsor suggesting side-effects, contraindications, and ineffectiveness in use of such components: Such summary should include an adequate bibliography of publications about the components and may incorporate by reference any information concerning such components previously submitted by the sponsor to the Food and Drug Administration. Include a statement of the expected pharmacological effects of the combination.

d. If the drug is a radioactive drug, sufficient data must be available from animal studies or previous human studies to allow a reasonable calculation of radiation absorbed dose upon administration to a human being.

16. A statement that all nonclinical laboratory studies have been, or will be, conducted in compliance with the good laboratory practice regulations set forth in Part 58 of this chapter, or, if such studies have not been conducted in compliance with such regulations, a statement that describes in detail all differences between the practices used in conducting the study and those required in the regulations.

If you have not conducted such investigations, i.e., item #6, could you state this in a letter. However, if you know of any company which has conducted these mechanical investigations, would you bring this to my attention so I can request the information from them.

Sincerely,

Norman Tinanoff, D.D.S., M.S.
Associate Professor
Department of Pediatric Dentistry

NT/1

Copy of labeling that will be used on the drug:

STANNOUS FLUORIDE IN POLYCARBOXYLATE CEMENT

Contents: 70% SnF_2 in polycarboxylate powder (W/W)

Caution: 10 grams of this product contains 1.7 grams of fluoride
                which approaches a lethal dose.

Directions: To be mixed with polycarboxylate liquid and placed into a
                tooth cavity preparation by a dentist as per protocol,
                "Clinical Trial with Controlled Release SnF_2".

THE UNIVERSITY OF CONNECTICUT

HEALTH CENTER

March 26, 1982

James P. Mann, M.D.
Director, Division of Surgical-
 Dental Drug Products
Food and Drug Administration
Rockville, MD 20857

Dear Dr. Mann:

   In accordance with the Code of Federal Regulations regarding Investi-
gational Exemption for New Drugs, I will:

   1. Notify the F.D.A. if investigations on controlled release SnF_2
       are discontinued.

   2. Notify each investigator if a new drup application is approved,
       or if the investigation is discontinued.

   3. Give full explanation why sale of a drug is required if the
       drug is to be sold.

   4. Not initiate human studies before 30 days after the date of
       receipt of this notice by the Food and Drug Administration,
       and I will continue to withhold clinical studies if requested
       to do so by the F.D.A.

                                 Sincerely,

                                 Norman Tinanoff, D.D.S., M.S.
                                 Associate Professor
                                 Department of Pediatric Dentistry

NT/1

PROPOSAL FOR A CLINICAL TRIAL

WITH CONTROLLED RELEASE SnF_2

Norman Tinanoff, D.D.S., M.S.

Principal Investigator

for

U.S. Army Institute of Dental Research

INTRODUCTION

The effective delivery of antimicrobials as well as other chemotherapeutic agents for the prevention or treatment of bacterial infections of tooth surfaces may be suboptimal due to its reliance on patient cooperation. Conventional methods for delivering of these agents to the oral cavity involve use of mouthrinses, gels, and dentifrices (Ainamo, 1977), and these systems are compromised in varying degrees due to their reliance on patient cooperation for repeated applications of the chemotherapeutic agent (Mirth and Bowen, 1976).

Interest in sustained release systems for drug delivery in medicine and dentistry has been increasing. Besides taking the repeated administration of a drug away from patient responsibilities, controlling the rate and site of release may be a more effective means of administering a drug. To date, sustained release systems in dentistry have been explored for delivery of steroids for the management of apthous ulcers (Yeoman, Greenspan, and Harding, 1978); anti-fungal drugs for the management of denture stomatitis (Douglas and Walker, 1973; Thomas and Nutt, 1978); antibacterials for the control of plaque (Addy, 1981) and fluorides for the control of dental caries and remineralization (Mirth and Bowen, 1976; Duperon and Jedrychowski, 1980; Forsten, 1976; Zity, Gedalia, and Grajower, 1981; Whitford et al., 1980; Friedman, 1980; Mirth et al., 1981; Abrahams et al., 1981). To date, the largest clinical study has been performed with a trilaminate methacrylate sodium fluoride-releasing device cemented to the buccal surfaces of the teeth of 11 subjects. The intraoral device was found to elevate the levels of fluoride in plaque, saliva and urine, but had no effect on plaque or gingival parameters (Mirth et al., 1981).

Fluoride ions may act as a therapeutic agent by altering bacterial metabolism (Hamilton, 1977) as well as reacting physicochemically with

enamel to reduce enamel solubility or remineralize initial caries (for review, see Mellberg, 1976). Yet only stannous fluoride has been shown to reduce the quality of plaque at concentrations compatible with frequent oral use (for review, see Tinanoff and Weeks, 1979). Based on its demonstrated ability to inhibit plaque, stannous fluoride has been chosen as the active agent whose effectiveness might best be enhanced by incorporation into a sustained release delivery system, and the depot for the SnF_2 is an intra-coronal (tooth) preparation where the SnF_2 is mixed with a dental cement and used as a temporary restoration.

Pilot studies examining the physical and clinical properties of an intracoronal sustained release fluoride delivery system have been performed on one subject. Plaque scores decreased during the experimental period suggesting that the released SnF_2 affected bacterial growth or attachment. The SnF_2-polycarboxylate cement was an adequate temporary restorative material without significant side effects.

The purpose of this proposal is to perform two human clinical trials to investigate the microbiologic, clinical effects, and safety of the slow released SnF_2-delivery system.

Research Leading to Proposal

In Vitro Microbiology

A series of in vitro experiments have been performed to determine whether fluoride at concentrations compatible with slow release and human consumption could effect bacterial viability growth, acid production, glucan, and DNA formation. SnF_2 had more potent bacteriostatic and bactericidal effects than $SnCl_2$, NaF, Na_2SnF_6, or TiF_4. SnF_2, $SnCl_2$, Na_2SnF_6, and NaF (at 10 ppm F or Cl) reduced the growth yield of S. mutans, while acid production by this organism appeared to be reduced only in the fluoride-supplemented media. Bacterial growth in fluoride-supplemented media resulted in greater net amounts of both the water-soluble and alkali-soluble glucans per bacterial mass, with SnF_2 having the greatest effect, increasing the water-soluble component 10 times and the alkali-soluble component 3 times over the controls. Greater tin uptake was noted by cells exposed to SnF_2 than by those exposed to $SnCl_2$.

In Vitro Physical Properties of SnF_2-Polycarboxylate Cement

Control samples of polycarboxylate, zinc phosphate, IRM and zinc oxide eugenol, i.e., those without addition of SnF_2, showed compressive strengths of 23.0 ± 1.3, 14.1 ± 2.7, 5.3 ± 1.5, and 0.7 Klbs/in^2, respectively. The compressive strengths of the cements were decreased linearly with addition of SnF_2 to the powder component of the cement. Yet, polycarboxylate cement still maintained relatively high compressive strength even with large additions of fluoride (Figure 1). Zinc phosphate cement appeared to be more detrimentally affected by the SnF_2 than polycarboxylate cement. IRM and zinc oxide eugenol had initial low compressive strengths and the addition of SnF_2 inhibited the setting reaction to the extent that these materials were made unsuitable for further preparation.
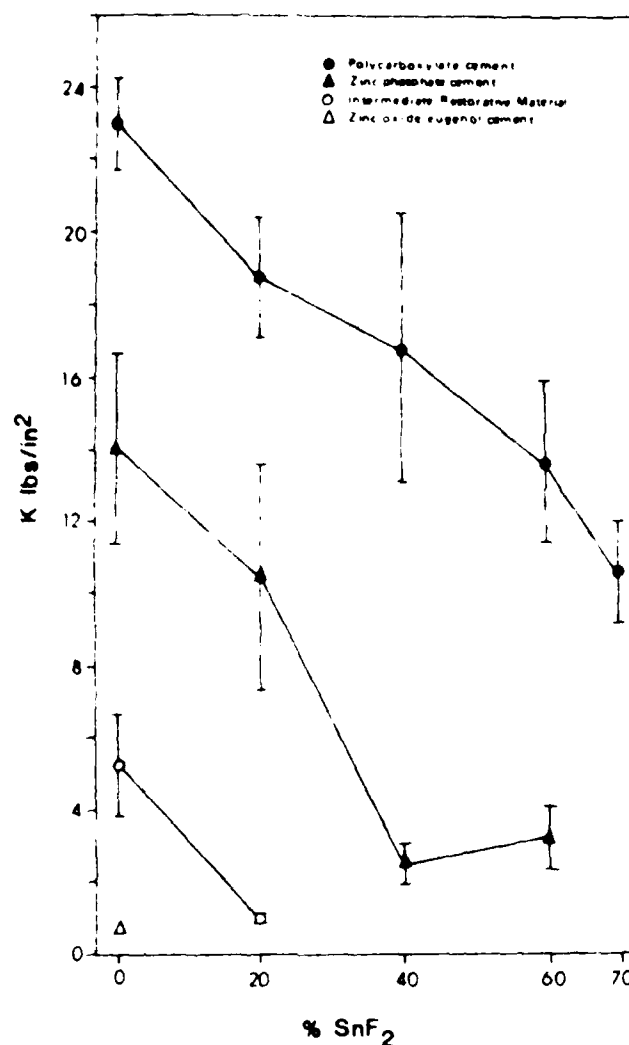
Figure 1:   Ultimate compressive strength (mean $\pm$ S.D.) of 4 dental cements
            containing from 0 to 70% SnF$_2$.

Release of fluoride from the SnF$_2$-cement mixtures showed that 70 percent

SnF$_2$ in polycarboxylate cement had the highest release of fluoride over 30

days with a mean of 3.7 $\pm$ 2.8 ppm F/day.   The release of fluoride from both

polycarboxylate and zinc phosphate cements was rather consistent each day.

In all cases, greater fluoride release was found in polycarboxylate cement

versus zinc phosphate concentrations of SnF$_2$ (Figure 2).   The mean fluoride

release from the IRM and zinc oxide eugenol cements was low, ranging from

0.1 to 0.4 ppm F/day.
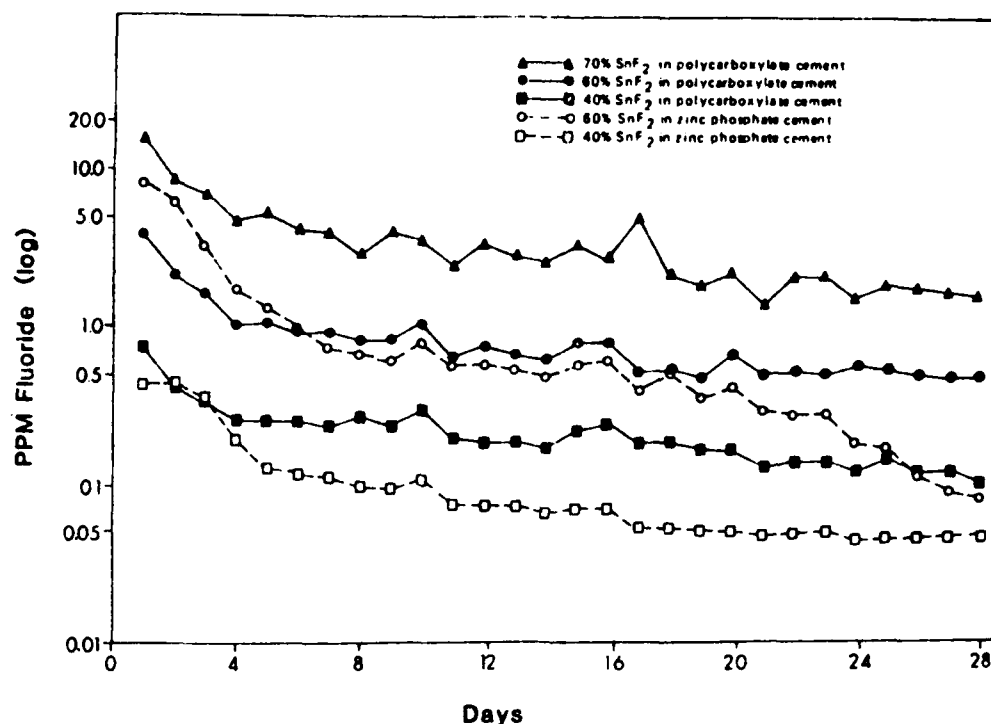
Figure 2: In vitro release of fluoride from 2 dental cements containing
40 to 70% SnF$_2$.

In Vivo Trial on One Subject

The baseline scoring, i.e., no oral hygiene for 2 days without SnF$_2$
temporary in place, showed a "total" plaque score of 3.5 ± 0.08 and a globu-
lar plaque score of 2.28 ± 0.58. During the experimental month, the mean
total plaque score was 2.9 ± 0.43 and the mean glubular plaque score was
0.96 ± 0.25. In the month following the experimental period, total plaque
returned to baseline levels; whereas globular plaque displayed a small
"carry over" effect (Figure 3).
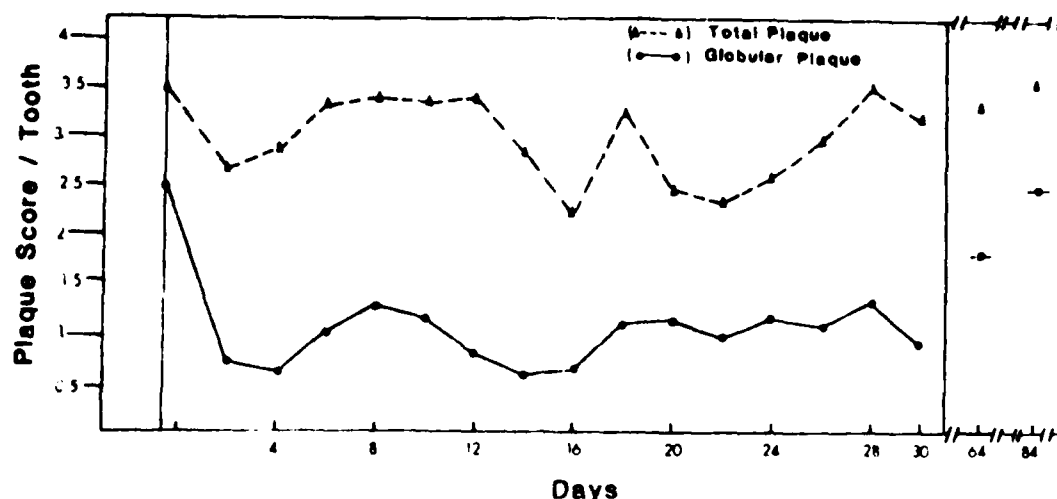
Figure 3: Visual plaque (total and globular) scores from subject during the 30 day period with the sustained release fluoride restoration in place and at approximately 1 and 2 months after the restorations had been removed.

The pre-experimental baseline for salivary and urinary fluoride were 0.039 $\pm$ 0.015 and 1.6 $\pm$ 0.5, respectively. The mean salivary fluoride level, during the experimental month, was increased to 1.86 $\pm$ 1.32 ppm F with the greatest elevation in the first 2 weeks. The urinary fluoride levels peaked in the first 2 days and returned to normal daily fluctuation after the first week (Figure 4). The relationship between the elevation in salivary fluoride level and the reduction in the glubular plaque score was nonlinear as evidenced by the weak correlation coefficient (r = -.3).
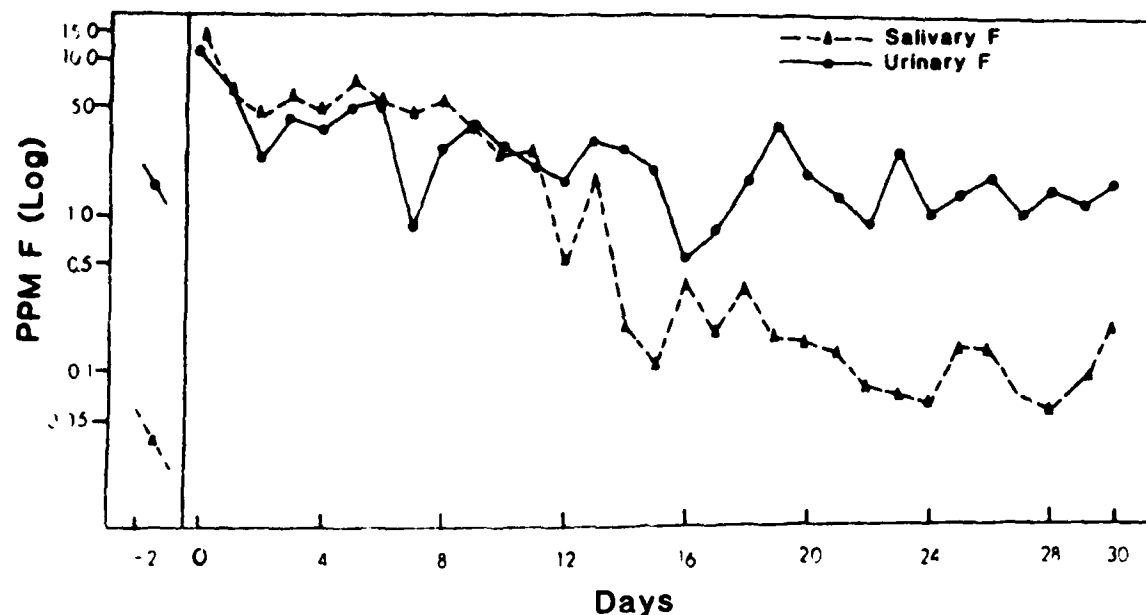
Figure 4: Daily fluoride concentration in saliva and urine from subject, in the 2 day baseline period and in the 30 day period with the sustained release fluoride restoration in place.

Clinically, the SnF_2-polycarboxylate restoration had no unfavorable properties in the one month trial. Aside from the staining of the tongue, no local or systemic side effects were noted. Moreover, the integrity and wear of the restoration was not significant. The intracoronal site of release allowed for good retention while not being bulky. The disadvantage of the location is that a patient must have a carious lesion or defective restoration in a tooth that can be used for the site prior to placement of a permanent restoration. Based on the favorable release of fluoride, mechanical properties, and putative antiplaque properties of the SnF_2-polycarboxylate temporary restoration, clinical trials using microbiologic as well as clinical parameters are indicated to assess the feasibility of this system as an adjunct in the control of caries and periodontal disease.

Material and Methods

Subjects

For the purposes of examining the short term clinical effect and length of efficacy of controlled release SnF_2, 20 dental students will first be used in a cross-over-design trial of 2 week experimental periods, and then later 8 students will be used in a longer term (approximately 2 month) trial. Those dental students who have at least one large defective amalgam restoration in a molar and over 100,000 $\underline{S.mutans}$/ml saliva will be asked if they are interested in participating in such studies. The informed consent of all subjects will be obtained after the nature of the procedures and risks have been fully explained. Students who have appropriate oral conditions and sign the consent form will have the defective restoration replaced temporarily with either 70% SnF_2-polycarboylate cement or polycarboylate cement alone. At the end of the trial all test teeth will be optionally restored with either silver amalgan or gold as needed. Subjects in the long term study will have to be available daily for collection of urine and saliva for fluoride analysis; and for one half hour weekly for clinical index recording as well as plaque and saliva collection. (Since accuracy of urine and saliva for fluoride analysis is not dependent on time, subjects will be issued vials for self collection on weekends and school holidays).

From the initial trials in the one subject, estimated fluoride lost (swallowed) from the restoration over the 1 monthly period was 57 mg. This level of fluoride consumption approximately 2 mg per day, is safe for adults (Forrester and Schulz, 1974). Tin consumption, on the other hand, is non-toxic to humans, and as much as 420 mg/day is eaten in canned foods (Christian-Feldman, 1970), a level well above that which could be consumed due to the release of tin from the restoration. Hence, the risk to adult human subjects

due to the injestion of SnF_2 in such a trial is negligible. The only risks associated with the proposed trial is believed that which would be incurred doing the necessary routine dentistry to the subjects.

Experimental Design for Short Term Trial

Twenty subjects will be divided into 2 groups systematically with regard to the level of S. mutans/ml saliva. That is subjects will be screened initially and ranked according to their levels of S. mutans/ml saliva and then alternately assigned into treatment or control groups so that the mean S. mutans per subjects will be similar in both groups.

The 2-1 week trials will include an initial preparation period of 7 days, two sequential "experimental" periods, each of 7 days, and an interim recovery period of 14 days between experimental periods 1 and 2. At the start of the initial preparation period, the teeth of all participants will be thoroughly scaled and polished with a non-fluoride pumice paste to remove all calculus and plaque. Detailed instructions in effective techniques of toothbrushing and flossing will also be given to ensure good plaque control during the 7-day initial preparation period and during the 14-day interim recovery period.

On day 1 of experimental period 1, i.e. at the start of the experiment, each subject will again have stimulated saliva collected to determine the number of total colony forming units and the number of S. mutans/ml saliva. Then the Gingival Index (GI, Löe & Silness 1963) will be recorded on the teeth (16, 21, 24, 36, 41, 44) identified as representative of the whole dentition (Ramfjord 1959). To insure that the teeth were free of plaque prior to implementing the mouthrinse regimen, the teeth will be stained with disclosing solution (5% fast green) and all visible deposits removed. Fast green will be used as disclosing solution because it has been reported to have no inhibitory

effect on microbial growth (Caldwell & Hunt 1969). The one defective restoration which had previously been identified will be removed and replaced by either 70% SnF_2 in polycarborylate cement (experimental) or polycarborylate cement alone (control). Teeth that have restorations involving proximal surfaces will have an orthodontic band cemented around it prior to restoration with the temporary. All cavity preparation will have the pulpal floor lined with a calcium hydroxide base prior to placing the restoration. Prior to the patient being dismissed the subjects will be instructed to abstain from all forms of active oral hygiene for the next 7 days.

At the end of experimental periods 1, clinical data (GI, PS) will be recorded, a saliva sample will be obtained, and plaque samples will be collected from the six previously selected teeth. The Plaque Score (PS) will be recorded, after staining the teeth with fast green (5%), according to a method described by Martens & Meskin (1972). Then all supragingival plaque present on the test teeth will be collected with a sterile carver. Prior to the plaque collection teeth will be dried with air and isolated with cotton rolls to minimize contamination of the samples by saliva. The plaque from each subject will be immediately placed on a preweighed, sterile aluminum carrier, which will then placed in a glass vial. To prevent excessive drying of the sample, each vial will contain a cotton pellet saturated with water. The vial will be taken to the laboratory where the wet weight of the plaque sample will be obtained within 5 min of collection.

Microbial examination of the saliva sample will be performed by the method of Klock and Krasse, 1977. In this technique one ml of saliva will be serially diluted and plated on mitis salivarius bacitracin agar, for S. mutans counts; and blood agar for total colony forming unit counts.

At the end of experimental period 1, the temporary restoration will be removed from all subjects and replaced with Intermediate Restorative Material. The subjects will also resume plaque control during this 2 week interim period. At the start of the second experimental period, each subject will again have a saliva sample collected, scored for gingival health, made plaque free, and have the temporary restoration replaced with either 70% SnF_2 in polycarboxylate cement or polycarboxylate cement alone according to the cross-over design, i.e., those subjects in the experimental group initially will be in the control group and vice versa.

At the end of experimental period 2, clinical data, saliva and plaque will again be obtained identically to that collected in the first experimental period. Since this design allows for each participant to act as his own control (providing that there is no carry-over effect), the paired t-test will be used for the statistical analyses.

Experimental Design for Two Month Trial

A "time series" approach, i.e., periodic measurement of individuals with introduction of a variable (SnF_2) into the time series will be the approach used to study: (1) the effect of slow released SnF_2 on salivary and urinary fluoride levels, (2) the correlation of salivary fluoride levels and antimicrobial activity, (3) the length of antimicrobial effectiveness of the restoration, (4) the clinical integrity of the restoration, and (5) side effects.

In this study salivary and urinary fluoride levels will be measured daily for 7 days on 8 dental students. Fluoride will be determined by fluoride specific electrode after samples are buffered with TISAB. One ML of the saliva will also be used for baseline quantitation of each subjects total aerobic and S. mutans levels. (Klock & Krasse, 1977) On day 7 each subject will have

the SnF_2 - containing temporary restoration placed in one molar. In the next 42 days each subject will be sampled to determine salivary fluoride, urinary fluoride, total aerobic and $\underline{S}$. mutans per ml saliva.

On day 42 the SnF_2 -containing temporary restorations will be removed and a polycarboxylate "only" restoration will be placed in the tooth cavity for 7 more days. The students will continue to be sampled during this time for the post-experimental baseline and possible carry-over effect.

Each week the restoration will be examined clinically and photographed. Any restoration that has failed will constitute the end of the variable period for that subject. The SnF_2 containing restoration would then be removed at this point and a baseline 1 week period for the subject would be initiated

This time series clinical trial, while not permitting for traditional statistical analysis of data, will allow for relatively long term observation and correlation of measurements. The effect of the slow release SnF_2 on oral microbiology over time can be analyzed for each individual as well as for the group. Correlations of salivary aerobic and $\underline{S}$. mutans levels to salivary fluoride levels will be easily obtainable. Alterations in urinary fluoride levels, an undesirable effect, can also be assessed and correlations to salivary fluoride levels.

References

Abrahams, L. J., Yonese, M., Higuchi, W., Fox, J. L., Charbeneau, G. T. (1970) In vivo remineralization using a sustained topical fluoride delivery system. Journal of Dental Research, 59, 583.

Addy, M.: (1981) In Vitro studies into the use of denture base and soft liner materials as carriers for drugs in the mouth. Journal of Oral Rehabilitation, 8:131.

Ainamo, J. (1971). Control of plaque by chemical agents. Journal of Clinical Periodontology, 4:23.

American Dental Association Specification No. 8 for dental zinc phosphate cement. (1969) Journal of the American Dental Association, 74:1566.

Caldwell, R. C. and Hunt, D. E. (1969) A comparison of the antimicrobial activity of disclosing agents. Journal of Dental Research, 48:913.

Christain, G. D. and Feldman, F. J. (1970) Atomic Absorption Spectroscopy. Application in Agriculture, Biology, and Medicine. Wiley-Interscience, New York, Page 403.

Douglas, W. H. and Walker, D. M. (1973) Nystatin in denture liners, an alternative treatment for denture stomatitis. British Dental Journal, 135:55.

Duperon, D. F. and Jedrychowski, J. Release and enamel uptake of fluoride from a fluoride-containing polycarboxylate cement. (1980) Journal of Pedodontics 4:287.

Forester, D. F., and Schulz, E. M. (1974) International Workshop on Fluorides. University of Maryland, Pages 105-106.

Forsten, L. (1976) Fluoride release from a fluoride-containing amalgam and two luting cements. Scandanavian Journal of Dental Research, 84:348.

Hamilton, I. R. (1977) Effects of fluoride on enzymatic regulation of bacterial carbohydrate metabolism. Caries Research, 11 (Suppl. 1):262.

Klock, B., and Krasse, B. (1977) Microbial and salivary conditions in 9-to 12-year-old children. Scandinavian Journal of Dental Research 85:56.

Löe, H. and Silness, J. (1963) Periodontal disease in pregnancy I. Prevalence and severity. Acta Odontologica Scandinavica, 21:533.

Martens, L. V. and Meskins, L. H. (1972) An innovative technique for assessing oral hygiene. Journal of Dentistry for Children, 39:12.

Mellberg, J. R. (1977) Enamel fluoride and its anti-caries effect. Journal of Preventive Dentistry, 4:8.

Mirth, D. B., and Bowen, W. H. (1976) Chemotherapy: antimicrobials and methods of delivery. Proceedings - Microbial Aspects of Dental Caries. Eds., Stiles, H. M., Loesche,W. J. and O'Brien, T. C., Special Supplement of Microbiology Abstracts, 1:249.

Ramfjord, S. P. (1959) Indices for prevalence and incidence of periodontal disease. Journal of Periodontology, 30:51.

Tinanoff, N., and Weeks, D. B. (1979) Current status of SnF_2 as an anti-plaque agent. Pediatric Dentistry, 1:199.

Thomas, C. F., and Nutt, G. (1978) The in vitro fungicidal properties of viscogel, alone and combines with nystatin and amphoterocin B. Journal Oral Rehabilitation, 5:167.

Whitford, G. M., Ekstrand, J., Callan, R. S., and Pearson, D. E. (1980) Subcutaneously implanted alginate as a continuous release source of fluoride. Journal of Dental Research, 59:186.

Yeoman, C. M., Greenspan, J. S., and Harding, S. M. (1978) Recurrent oral ulceration. A double-blind comparison of treatment with betamethasone valerale aerosol and placebo. British Dental Journal, 144:114.

Zity, A., Gedalia, I., and Grajower, R. (1981) Addition of fluoride compounds to acrylic resin plates: binding strength and fluoride release. Journal of Oral Rehabilitation, 8:37.

STUDY 1: DETERMINANTS OF THE ANTIBACTERIAL EFFECTS OF SNF$_2$
AGAINST S. MUTANS: IONS, pH VALENCE

Introduction

Fluoride compounds have been used topically in the oral cavity for many years with the intention of affecting tooth enamel to alter its resistance to dental caries. Fluoride may also affect the bacteria in the mouth. Bibby and van Kesteren (1942) found that 1 ppm F$^-$ as NaF reduces bacterial acid production. While there are some antibacterial properties of NaF, SnF$_2$ has been shown to have significantly more affect against oral microorganisms in vivo and in vitro. Recently SnF$_2$ has been shown to selectively reduce S. mutans, the bacterium associated with dental caries.

The difference between NaF and SnF$_2$ in affecting oral bacteria have been suggested to be due to: 1.the divalent cation ,Sn, competing with calcium to alter bacterial adhesion/cohesion; 2. tin oxidizing the thiol groups of bacterial enzymes; 3. the large uptake of tin disrupting bacterial metabolism; or 4. the naturally low pH of SnF$_2$ which would allow HF formation and thus be more antibacterial.

The purpose of this report was to compare SnF$_2$ to other compound having similiar ions, pH, valence, or atomic weights to determine what characteristic of SnF$_2$ is necessary to produce the apparent antibacterial affect against S. mutans.

114

Materials & Methods

Microorganism and Medium

A streptomycin-resistant mutant of _Streptococcus mutans_ NCTC 10449, known to adhere to smooth surfaces _in vitro_, and to produce dental caries in rats was used in this study. Stock cultures were maintained by monthly transfer in fluid thioglycollate medium (Difco) supplemented with 20% v/v meat extract and excess $CaCo_3$. For experiments, cultures were adapted and grown in the complex medium of Jordan et al. (1960), supplemented with 50 mg of Na_2CO_3/L and containing 5% sucrose (pH 7.5).

Experimental Design

Stainless steel wires (0.5 mm diameter), suspended in culture tubes by rubber stoppers, were used as a substratum for the bacterial plaques. For plaque growth, 10 ml of the complex medium was inoculated with 0.1 ml of the adapted _S. mutans_ cultures and the wires were initially colonized by inoculating them in the medium for 12 hr. at 37^0. Intermittent exposure to the various test agents (Table I), starting after 12 hr. growth, involved removing each wire from the medium, placing them into the appropriate test solution for 1 min., reducing carryover of test solution with a 1 min. non-agitated rinse in 10ml H_2O (pH 6.0), and then placing the wire into 10 ml of fresh growth medium. This exposure of the adherent plaques to the test solutions was repeated 2 more times at 12 hr. intervals.

All experiments were terminated after 48 hrs., 12 hrs. after the last exposure to the test agents. The thickness of the adherent plaques was visually scored by comparing the growth to standards. Except for those samples processed for electron microscopy, the plaques from each wire were

collected into pre-weighed tubes, pelleted by centrifugation, dried for 3 days at 70°, and weighed. The pH of the growth medium representing 12 hr. growth was also determined at the end of the experiments.

An exception to the above procedure took place in one trial the purpose of which was to test the effect of agents on "pre-formed" plaque. In this experiment, the only difference was that plaque was allowed to form on the wires for 48 hours before they were exposed to the test agents. In this case, the experiment was terminated on the 4th day.

Atomic Absorption Spectrophotometry

Dried samples were further processed to determine their metal content. Tin quantity was determined by an atomic absorption using a spectrophotometer (Perkin-Elmer, Model 403) equipped with a graphite furnace (Model HGA-74). Lead was also determined with the aid of the graphite furnace. Zinc and copper was quantified by the method of addition using flame atomic absorption spectrophotometry. A deuterium lamp was used in all cases to correct for non-atomic absorption signals.

Transmission Electron Microscopy

Bacterial specimens designated for electron microscopy were fixed on their wires at the end of the experiment (48 hr) with 2.5% gluteraldehyde in phosphate buffer (pH 7.4, 390 mOsm) and postfixed in 1% osmium tetroxide in veronal buffer (pH 7.3). The fixed bacteria were then removed from the wires, washed in phosphate buffer, dehydrated in acetone and embedded in epoxy medium. Thin sections were prepared with a LKB ultramicrotome using a diamond knife. Silver-gold colored sections were examined unstained with a Zeiss EM10 electron microscope at 80 kV.

Energy-dispersive x-ray analyses, to confirm the presence of specific

metal deposits in or on the bacteria, were performed in a JOEL JEM-100 CX

transmission electron microscope equipped with a high resolution electron

microscope accessory (ASID) and a Kevex SI (LI) x-ray detector connected to

a Micro-X Analytical X-ray Spectrophotometer, Model 7000.

Results

An initial trial (Table 2), comparing the intermittent exposure of four fluoride containing solutions at their natural pH's showed that SnF_2 had several effects on the growth of adherent S. mutans cultures compared to the other fluoride solutions. Obvious differences in plaque scores, dry plaque weights, and acid production were found on all the wires exposed to SnF_2. No differential growth was noted in those plaques exposed to NaF, ZnF_2 or SnF_4. The metal content of the dried plaques was also variable among the treatment groups. There was little metal content in those samples exposed to ZnF_2. Those samples exposed to SnF_4 had tin present, but not nearly as great as those plaques exposed to SnF_2. In the SnF_2 samples, approximately 4% of the plaque dry weight could be attributed to tin.

Another trial was performed in an attempt to discriminate whether the pH, the fluoride, or the tin content could account for the noted growth inhibition of the SnF_2 solution (Table 3). In this trial, even though SnF_2 at pH 2.5 had antibacterial effects, there was no growth alteration apparent due to fluoride, pH or due to tin, per se. The pH of the SnF_2 solution was critical. Since the SnF_2 solution adjusted to pH 7.0 had no effect.

To further understand the effect that the pH of SnF_2 had on the observed antiplaque properties, SnF_2 was prepared with in a range of pH's from 2-6, and tested in the same bacterial growth system (Table 4). SnF_2 appeared to have more growth inhibition at lower pH's, with those plaques exposed to SnF_2 below pH 4 being most affected. Besides the low pH of SnF_2 solutions producing the most growth inhibition, plaques exposed to these solutions had the most metal content per mg plaque (Table 4, Figure 1).

118

Two other metallic fluoride compounds were also compared to NaF to examine whether other compounds would have similar effects to SnF_2. Neither PbF_2 or CuF_2 at the 2 pH's examined showed any effect more than NaF at comparable pH levels (Tables 5 & 6).

The possibility was also explored that established bacterial colonies would affect the antibacterial properties of SnF_2. S. mutans plaques were allowed to preform on the wires for 2 days before being exposed to SnF_2. While there was some reduction in visual plaque score and acid production in those samples exposed to SnF_2, no difference in plaque weight was noted after the weight of tin was subtracted from the plaque dry weight (Table 7).

Electron micrographs of those bacterial specimens exposed to H_2O, NaF, $SnCl_2$, $ZnCl_2$ and all appeared similar with morphologically normal gram-positive cocci surrounded by extracellular material. However, the bacteria exposed to SnF_2 had frequent electron-dense granules, most often within the bacterial cell, but occasionally on the outer cell wall. Energy-dispersive x-ray microanalysis of this electron dense material revealed peaks corresponding to the $L\alpha$ (3.67 KeV) and $L\beta$ (3.44 KeV) peaks for tin. Besides the electron dense areas, the bacteria exposed to SnF_2 frequently were also noted to have intracellular electron-lucent holes and distorted all shapes (Figure 3). A unique feature of the specimens exposed to lead fluoride was the presence of electron dense granules located only on the outer cell wall (Figure 4). Electron microprobe of these granules confirmed them to be lead (L = 10.50, L = 12.62 KeV).

119

Discussion

The results in these series of experiments confirm our earlier findings that SnF_2 has antibacterial properties against S. mutans, and this effect appears related to the uptake and retention of tin by this organism. However, the present studies show that compounds similar to SnF_2 in pH, ions, or valence have little or no effect on S. mutans growth or colonization.

While others have reported that an acidic solution of NaF has some antibacterial properties due to the cellular uptake of hydrogen fluoride, our in vitro model using intermittent exposures to test agents showed only slight reduction of acid production and no growth inhibition in those bacteria exposed to NaF at a low pH. The only apparent effect that pH alteration had on the test solutions was that noted with SnF_2. In all *growth parameters tested, there was an inverse relationship of the pH to* SnF_2 solutions and its effectiveness as an antibacterial agent. SnF_2 solutions near neutrality showed no antimicrobial properties. Hydrolysis of SnF_2 solutions at elevated pH's probably results in the reduced antibacterial properties.

The degree of bacterial colonization of the wires at the time of the initial SnF_2 exposure was also an important parameter in the effectiveness of SnF_2. In our experiments, we allowed 12 hours for the wires to become colonized by S. mutans before the first exposure to the test solutions. Bacteria that had less time to colonize the wire had essentially no growth after the exposure to SnF_2. Conversely, heavy bacterial colonization of the wires, as with preformed plaque, reduced the effectiviness of the 1 minute exposures to SnF_2. Preformed plaques would not visually grow, but they still were metabolically active as shown by their ability to reduce

120

the pH of the growth medium. The decreased effectiveness of SnF_2 with preformed plaques agrees with previous findings *in vitro* and *in vivo*. Perhaps a thick bacterial mass reduces diffusion of this antibacterial agent.

Unexpected in this study was the finding that only SnF_2 showed antibacterial properties against *S. mutans*. Metal salts, especially those with high atomic weights, are generally regarded to have antibacterial properties. $SnCl_2$, however, has been previously noted to have little antimicrobial activity. This compound is unstable in aqueous solutions which probably reduces the available tin to the bacteria. The fluoride salts of lead and zinc were tested because they, like SnF_2, are divalent cations, with PbF_2 having a greater atomic weight and atomic diameter than SnF_2, and ZnF_2 being lighter and smaller. Although plaques exposed to PbF_2 in this study had quantative metal uptake, the electron micrographs showed the lead to be located only on the outer cell wall. No alteration in bacterial growth parameter due to PbF_2 was observed. Other reports have also found that bacterial cell membranes can bind lead with no detectable effect on growth. ZnF_2 also showed no effect on bacterial growth, but in contrast to PbF_2, no zinc binding in or on the bacteria was noted. Other investigations have shown little inhibition of bacterial growth by zinc compounds.

Surprisingly, no effect on bacterial growth was noted for CuF_2 or SnF_4. CuF_2 has been reported to be effective in reducing bacteria acid production *in vivo*. In the present study, there was no measurable reduction in bacterial acid production by CuF_2, nor did CuF_2 alter any other bacterial growth parameters. SnF_4, although showing no effect on the growth of *S. mutans*, produced some quantative retention tin in the cells. The electron

121

micrographs, however, showed that with SnF_4, the electron dense deposits were located only on the surface of the cells.

The apparent unique antibacterial properties of SnF_2 thus appears to be assiciated with the observed intracellular retention of tin. The high percentage of tin in the bacteria as measured by atomic absorption spectrophotometry and the large numbers of intracellular tin deposits found in those bacteria exposed to SnF_2 suggests that S. mutans cells in some way transports this metal into the cell where it becomes firmly bound. The apparent condensation of the tin into intracellular granules may be an attempt by the bacteria to reduce the harmful effects of this foreign ion. Metalic granules have also been found in eucaryotic cells exposed to metal compounds.

The intracellular tin accumulation, even though in granules, still appear to disrupt S. mutans metabolism as demonstrated by the reduced growth, acid production, and the electron microscopic presence of holes in many of the bacterial cells. The intracellular holes found in the SnF_2 treated specimens may be a sign of unbalanced bacterial growth.

The unique antibacterial properties of SnF_2 against this test organism is still not fully understood. No single property of SnF_2 which could be isolated (i.e., pH, valence, cations, or size of the molecule) was identified as an important parameter necessary for its effectiveness. SnF_2 in aqueous solution is highly reactive and the chemistry of the reactions are not completely understood. It might be that this reactivity or one of the species formed in solution is the important variable which allows for the uptake of tin into the S. mutans cell and the consequent antimicrobial properties.

122

CONCLUSION

Twice daily exposure of adherenct $\underline{S.}$ $\underline{mutans}$ to various fluoride salts has shown that only Snf_2 significantly alters thais organisms growth and metabolism. The antibacterial affect of SnF_2 was associated with an uptake of tin into the bacteria. Fluoride salts of sodium, lead, zinc, and copper had little effect in this test system. The pH of the various fluoride salts generally had no effect on the test compound activity except for the noted inactivation of SnF_2 at elevated pH's.

Since SnF_4 also did not effect the growth or metabolism of $\underline{S.}$ $\underline{mutans}$, a unique property of SnF_2, possibly it reactivity in an aqueous environment, may be responsible for it antibacterial properties.

STUDY 2: EFFECT OF NAF AND SNF$_2$ IN DRINKING WATER
ON HAMSTER DENTAL CARIES

INTRODUCTION

Besides the unquestionable dental caries reduction produced by fluoride ions, caries has also been shown to be inhibited in man and experimental animals by antiplaque agents. Several studies have demonstrated that, in addition to its well established physicochemical interactions with enamel, stannous fluoride may also possess antiplaque properties. A recent study has shown that the antimicrobial properties of SnF$_2$ can be demonstrated in vitro even as as low as at 10 ppm F$^-$.

There has been only one study in humans or experimental animals that has compared the caries reduction of SnF$_2$ to NaF. In that study, fluoride supplementation (18 ppm F$^-$) in water as SnF$_2$ produced a 78% caries reduction in rats, while NaF produced a 52% reduction. The present study was performed to reexamine whether SnF$_2$ produced greater caries reduction than NaF. Furthermore, our study was to examine whether the potential antibacterial effects of SnF$_2$ was correlated to a possible greater caries reduction of this compound in an experimental rodent caries study.

METHODS AND MATERIALS

Animals, Diet, Infection

This study was conducted on 45 "conventional" golden outbred Syrian hamsters. To insure that the effect of NaF and SnF_2 was essentially topical, the experiment was begun when the animals were approximately 38 days old. The Syrian hamsters third molars begin eruption on day 30 and occlusion is obtained between the 40-45 day.

On day 1 of the experiment the hamsters were orally inoculated with 0.2ml of a log culture of streptomycin resistant Streptococcus mutans NCTC 10449 in fluid thioglycollate medium; and given the NIH 2000 cariogenic diet and deionized water ad libitum. On day 2, the hamsters were reinoculated, and randomly distributed into deionized H_2O, NaF, and SnF_2 groups. Fifteen hamsters (5 per cage) received 5 ppm fluoride as NaF (0.0110 g/l, pH 6.5); another 15 received 5 ppm fluoride as SnF_2 (0.0207 g/l, pH 4.0); and the third group received water (pH 7.2). The fluoride solutions were made and distributed into plastic feeding bottles each day.

The diet and water continued to be supplied ad libitum until the animals were sacrificed on day 64. The animals were weighed at the beginning and at the end of the experimental period.

Recovery of Microorganisms

The left maxillary and mandibular molar teeth were used for microbiologic recovery of the streptomycin-resistant infectant. Consecutively, the hamsters were overdosed with pentobarbital, decapitated, and the cheeks and mandibular condyles were cut to enable better access to the jaws. The molar crowns, along with some supporting bone, were excised as a unit with a dental Rongeur and placed into 3 ml of cold 0.05% yeast

125

extract broth (pH 7.0). The organisms were dislodged from the teeth by sonication (Bronson Model W185D, Plainville, NY) using a microtip for 40 sec at 50 watts and an output setting of 4.

Serial dilutions were performed using the micro-method of Westergren and Krasse and plated on Mitis Salivarius agar supplemented with 0.001% potassium tellurite and 200 g/ml of streptomycin. After incubation in candle jars for 48 hr at 35°, the streptomycin-resistant _S. mutans_ were counted.

Caries Scoring

To assess caries, the right mandible and maxilla were defleshed by dermestid beetles. The numbers and extent of the fissure and smooth surface lesions were observed under a dissecting microscope and scored by the method of Konig, modified so that only enamel and dentinal carious lesions were differentiated. Enamel caries was defined as areas of the smooth surface or fissures which had opaque or chalky white areas in the enamel. Dentinal lesions were defined as obvious breaks in the enamel. Proximal surfaces were observed by slightly separating the teeth rather than slicing the teeth mesial-distally which could completely remove the lesions. The jaws were numerically coded so that the experimental histories were unknown to the scorer.

Statistical Analyses

Differences among group caries scores, microbiological recoveries, and animal weights were tested by analysis of variance using the Scheffe multiple comparison procedure. All tests were performed at the 0.05 level of significance.

RESULTS

All animals survived and appeared in good health at the end of the experiment. The mean weights and standard deviation per group in grams were: 158 ± 11, 148 ± 14, and 142 ± 9 for the H_2O, NaF, and SnF_2 groups, respectively. These weights were significantly different suggesting that the fluoride compounds may have had an effect on the weight gains during the experimental period. In 5 animals, minimal microbial recovery of S. mutans from the molars ($<10^4$) resulted in elimination of these animals further analysis.

Table 8 shows the microbial recoveries of the S. mutans at the end of the experiment. The recovery of strain 10449 was variable between animals and neither the actual mean nor the logarithmic transformation of the recovered numbers showed statistical differences between groups.

Table 8 also summarizes the caries data scores and Figure 5 illustrates the scores of enamel and dentinal caries found in the three groups of animals studied. The caries produced in the experiment were mainly small and uncoalesced enabling accurate scoring of the number of lesions. The majority of the carious lesions were of the pit and fissure type with only 11 smooth surface lesions being found. Ten of these smooth surface lesions were found in those hamsters in the deionized water group and one smooth surface lesion in the SnF_2 group. The reduction of enamel and dentinal caries in both the NaF and SnF_2 groups was significantly different from the deionized H_2O group. The two fluoride groups, however, were not statistically different in any caries parameters examined.

DISCUSSION

Certain characteristics of experimental caries in hamsters should be recognized when interpreting these results. Dental caries on the smooth

surfaces of rodents, like man, is dependent on the formation of adherent plaque. However, rodent sulcal lesions are not associated with plaque formation, and may be modified by the impaction of food and debris. Thus an agent used to reduce caries rate by altering microbial growth or adherance, as in the present study, would most likely have the greatest effect on the number of smooth surface lesions where it could exert antibacterial activity.

An example of an antiplaque agent being more effective on smooth surfaces caries is chlorhexidine gluconate, which in a rodent study has been shown to inhibit smooth surface but not sulcal caries. In the present study, essentially only those animals in the deionized water group had smooth surface caries. Therefore, it was not possible to differentiate a potentially greater caries reduction by SnF_2, due to its antibacterial properties, from the "fluoride only" effect of NaF on the smooth surfaces.

As expected, the present study also demonstrated that both NaF and SnF_2 reduced sulcal carious lesions. Several studies have shown that sulcal caries in hamsters and rats can be significantly reduced by the continuous administration of low levels of fluoride in the drinking water , or higher concentrations applied topically. Our results did not find SnF_2 to have a statistically greater effect than NaF on sulcal lesions. Besides the poor potential of antiplaque agents on rodent sulcal lesions, the concentration of the SnF_2 was possibly too low to have an antimicrobial effect. Antiplaque properties of SnF_2 at mouthrinse concentrations (100- 1,000 ppm F^-) are known to improve as the concentration of SnF_2 increases. Furthermore, very dilute solutions of SnF_2, as in the present experiment, may quickly lose soluble stannous ions. Low concentrations of chlorhexidine have also been found to be ineffective in reducing caries in rats. A low

128

concentration of SnF_2 (0.002%) was examined in the present because higher concentrations of fluoride ions (10 ppm F^- as NaF) have been shown to almost entirely eliminate rat caries which would further hinder the possibility of observing differences between SnF_2 and NaF.

Another characteristic of experimental rodent caries is the large variability associated with microbial recovery of the infectant. Our technique of excising the crowns in toto and recording the absolute bacterial recoveries rather than relative recoveries, was thought to potentially decrease experimental variance. Yet as with other studies, our data shows large differences in microbial recoveries even among animals in the same group. Possibly the variance is the result of true differences in infection among the animals rather than experimental error.

CONCLUSION

Stannous fluoride was compared to NaF (5 ppm F^-) in the drinking water of hamsters to test whether SnF_2 had greater caries inhibitory effects due to its potential antibacterial effects. Caries was produced in the hamsters by orally inoculating them with streptomycin-resistant S. mutans, and feeding them NIH diet 2000. After sacrifice on day 64, the hamster's left molars were used for microbial recovery and the right molars were used to assess caries.

The number of enamel and dentinal carious lesions in both the NaF and SnF_2 group was significantly different from the deionized water group; however, there was no difference in caries scores between the NaF and SnF_2 group. The recovery of S. mutans was highly variable between animals and showed no statistical difference between groups.

The present study demonstrated that both NaF and SnF_2 reduced sulcal and smooth surface lesions in the hamster, but the potential difference

129

between the fluoride compounds was not evident. It is possible that differences between NaF and SnF_2 cannot be shown in a rodent caries model due to: (1) the variability of infection and caries attack among animals; (2) the strong effect of fluoride ion on the caries rates; and (3) the necessary low concentration of the agents tested which may mitigate potential antiplaque effects.

STUDY 3: MICROBIOLOGIC EFFECTS OF SNF$_2$ AND NAF MOUTHRINSES IN SUBJECTS WITH HIGH CARIES ACTIVITY: RESULTS AFTER ONE YEAR

Introduction

The major efforts in the prevention of dental caries have been directed to treatment strategies that affect tooth enamel or to the development of agents that alter the cariogenic microflora. Fluorides, in various concentrations and regimens, have been the most successful agent to date in preventing this disease. Although the fluoride effect has traditionally been considered to be the result of its physicochemical interaction with enamel, there is evidence that fluoride also alters bacterial metabolism at low concentrations, and is bactericidal at higher concentrations. Chemical agents that solely affect bacteria (antibiotics and antiseptics) have also been shown to alter caries activity. Patients on prophylactic penicillin treatment for medical reasons have been noted to have reduced caries activity. Chlorhexidine, a potent antiseptic, has also been shown to reduce caries in children. Attempts to improve caries reduction by combining fluoride with antimicrobials have been only partially successful. Chlorhexidine diacetate (1%) combined with NaF (0.15%) has been shown to have an additive effect in reducing rat fissure caries, but the combination was not superior to each alone with regard to smooth surface caries.

It has recently become evident that a specific fluoride compound, stannous fluoride (SnF$_2$), has an antimicrobial effect at concentrations compatible with daily fluoride use. Short term clinical studies with SnF$_2$

131

have suggested possible important antimicrobial properties. There is presently no evidence to suggest that these antimicrobial properties would affect _Streptococcus_ _mutans_ and lactobacilli--bacteria correlated to the initiation and to the progression of dental caries in humans.

The aim of the present study was to compare the effects of mouthrinsing with NaF and SnF_2 on the number of total aerobic salivary bacteria, _S._ _mutans_ and lactobacilli numbers in a group of subjects screened for potential high caries activity.

MATERIALS AND METHODS

Subjects

The subjects in this study were adults over the age of 18 having incipient carious lesions, high numbers of unrestored carious lesions, and elevated numbers of salivary _S._ _mutans_. The 58 subjects were identified by their caries prevalence in the screening clinic at the University of Connecticut School of Dental Medicine. A follow-up microbial screening showed that 38 subjects had greater than 2.0×10^5 _S._ _mutans_ per ml saliva. These subjects were regarded as potentially high in caries activity. The 37 subjects who consented to the study were ranked by their recoverable number of salivary _S._ _mutans_ and then alternately assigned to a SnF_2 or an acidulated NaF mouthrinse group. During the first year, 15 patients withdrew from the study. Of the remaining 22 patients, 9 were considered partially compliant with the mouthrinsing procedures. Partially compliant subjects were those who, by their own report, missed more than 4 mouthrinses per month or who were inconsistent with mouthrinsing. We verified these reports by monitoring each patient's remaining supply of

132

mouthrinse and by questioning the patients monthly for recall of their fluoride usage.

Treatment

After baseline data were obtained, subjects were instructed to use 10 ml of their respective mouthrinses twice daily for 1 minute per rinse. The SnF_2 rinse was diluted with water (1 part rinse; 4 parts H_2O) immediately before use to produce a final fluoride concentration of 200 ppm F^- and a pH of 3.4. The acidulated NaF mouthrinse was used full strength at a fluoride concentration of 200 ppm F^- and a pH of 4.0. One month after the initiation of mouthrinsing, each subject received 3 dental hygiene appointments at one week intervals. The oral hygiene instruction, cleaning, scaling and root planing were performed by one dental hygienist, blind to the subjects' treatment categories. The subjects were also assigned to a dental resident for restoration of teeth with active carious lesions. They were contacted monthly to reinforce oral hygiene, to monitor their fluoride usage and to resupply them with mouthrinse.

Microbiology

Stimulated saliva, produced by chewing on a piece of paraffin wax, was collected from each subject at the screening, at the baseline examination, and after 1, 3, 6 and 12 months. Each saliva sample was sonicated for 1 min., vortexed for 30 sec., and serially diluted from 10^{-1} to 10^{-6}, in 0.05 M phosphate buffer (pH 7.0). From each dilution, 25 μl was spotted in duplicate on one-third of the surface of an agar plate. The dilutions having 20-100 colony forming units were counted with the aid of 20X magnification. The mean of the 2 samples from these dilutions was used in the analysis.

For cultivation of all aerobic bacteria, (Total CFU) dilutions were spotted on 10% sheep blood agar plates, incubated for 24 hours in a CO_2-

enriched environment (candle jar) at 35°C and counted. For determination of the number of S. mutans, a selective medium consisting of Mitis-Salivarius agar containing 0.2 units/ml Bacitracin was used. After spotting, the agar plates were incubated for 48 hours in a candle jar. Those colonies with morphologic characteristics of S. mutans were counted and their identities confirmed when necessary with biochemical tests. Lactobacilli were cultivated with the aid of Rogosa SL agar plates. The spots were allowed to dry and then a further portion of the agar was poured over the surface. Lactobacillus counts were determined after 48 hours incubation.

Statistical Methods

The difference in Total CFU, S. mutans, and lactobacilli between the two groups were analyzed by two way analysis of variance for repeated measures. Pairwise contrasts at each time interval were computed by the method of Schaffe. Due to unequal variances, logs of the microbial counts were used for this analysis. Differences in colony forming units between baseline and 1 year for each subject were analyzed by unpaired t-test. Subjects were also ranked according to levels of S. mutans and the difference between these ranked groups was analyzed non-parametrically by the Wilcoxon two-sample ranks test for unpaired measurements.

Results

Of the 38 subjects who started in the study, 22 remained after 1 year. Of these, only 13 were identified as being completely compliant with the instruction to rinse twice daily over the study year.

Analysis of Total CFU data showed no difference in this parameter between the NaF and SnF_2 groups (Table 9). Both groups, however, showed a significant two-fold reduction over baseline values in Total CFU after 1

134

year (Tables 9 & 13).

The alternate assignment of subjects by initial salivary $S.$ mutans levels into the acidulated NaF or SnF_2 groups produced two closely matched populations with regard to their mean numbers of $S.$ mutans and to their ranking relative to their $S.$ mutans levels (Tables 10 & 11). This match was maintained even though 40% of the subjects dropped out of the study. The mean recoverable salivary $S.$ mutans levels comprised 1.6% of the total recoverable flora in this population at baseline.

After 1 month the number of $S.$ mutans was significantly reduced from baseline in the group rinsing with SnF_2, especially in those subjects identified as completely compliant. At 3 months, the reduction of $S.$ mutans in these subjects showed maximum effect. Three subjects compliant with SnF_2 mouthrinsing had no detectable $S.$ mutans at this examination. At 6 months, there was a moderate rise in $S.$ mutans levels, which in the compliant subjects, leveled between from 6 months to 1 year. The subjects considered partially compliant with the SnF_2 rinsing regimen showed less dramatic reductions in $S.$ mutans counts until the 1 year examinations. At this point, a large drop in recoverable $S.$ mutans was evident (Table 10).

A significant difference in salivary $S.$ mutans levels between the SnF_2 and acidulated NaF subjects was evident at the 1 year exam for all degrees of compliance. The entire SnF_2 group at 1 year had dramatically lower $S.$ mutans counts from baseline levels, however, no change from baseline was detected in the NaF group (Tables 10 & 13). Of the 12 subjects in the SnF_2 group whose recoverable $S.$ mutans levels were greater than 2.0×10^5/ml saliva at baseline, 10 subjects were noted to have less than 200,000 $S.$ mutans/ml saliva at 1 year. The acidulated NaF group, showed no alteration in $S.$ mutans from their baseline levels (Table 11).

The lactobacilli recoveries showed no significant differences between

groups or longitudinally within the treatment categories. The variation in number of recovered lactobacilli was very large, even in the same subject at different sampling times. This lack of consistent recovery of lactobacilli is reflected by the standard deviations which were consistently higher than means at all sampling intervals (Table 12).

Discussion

Certain study design limitations should be recognized when examining the results and evaluating the appropriateness of the statistical tests. The 22 subjects remaining after 1 year do not represent a normally distributed population so these results should not be compared to the population in general. The population is skewed since only those subjects that had high caries prevalence and elevated salivary $S.$ mutans counts were selected as potential subjects, and only approximately half of the subjects who began the study were examined at 1 year. Instead of random assignment into the two treatment groups, subjects were ranked according to $S.$ mutans levels and then alternately assigned to rinse with acidulated NaF or SnF_2. This was done because $S.$ mutans levels, the most important variable, vary greatly among individuals and it was desirable to have this variable balanced at baseline due to the relatively few subjects. Furthermore, since the study has no real control group, the small but significant longitudinal change in Total CFU in both the treatment groups cannot be attributed with certainty to a treatment effect.

The change in $S.$ mutans counts are striking even considering the design limitations. SnF_2 appears to have a potent long term effect on salivary $S.$ mutans levels. While the effect is most evident in the patients identified as completely compliant with rinsing twice a day , it was also found in

those subjects who were partially compliant with mouthrinse usage. This
dramatic reduction in $S.$ $mutans$ may possibly explain why after 1 year the
subjects rinsing with SnF_2 had half the caries increment as those rinsing
with acidulated NaF.

In $vitro$ studies determining minimum inhibitory and lethal
concentrations of various fluorides have clearly shown that $S.$ $mutans$ is
more susceptible to SnF_2 than other fluoride compounds. The large tin
accumulation in bacteria exposed to SnF_2 may contribute to the greater
antimicrobial activity of SnF_2.

The apparent selective supression of $S.$ $mutans$ (reduction of $S.$ $mutans$
relative to Total CFU) by SnF_2 is also of interest, since $S.$ $mutans$ is now
considered the important microorganism for the initiation of dental caries.
Other human and animal studies using NaF, instead of SnF_2, have also shown
selective reductions in $S.$ $mutans$. Such selectivity of a non-antibiotic
against a specific pathogen is not easily understood. One in $vitro$ study
using minimium bactericidal concentrations found that $S.$ $sanguis$ and $S.$
$mutans$ had similar sensitivities to fluoride compounds[28]. Furthermore,
other effective antiplaque ꞊ꞌꞌꞌꞌents have shown only non-specific
antimicrobial properties against oral flora. The substantial supression of
$S.$ $mutans$ by SnF_2 found in this study and the reported selectivity of
fluorides in general against $S.$ $mutans$ needs further study. If SnF_2 does
have a specific effect against $S.$ $mutans$, then SnF_2 would have major
advantages over other agents in the treatment of subjects having high
caries activity associated with elevated $S.$ $mutans$ levels. The microbial
selectivity and the well documented physicochemical effect of SnF_2 on tooth
enamel would probably make this compound superior to sodium fluoride or
antiseptic treatment.

The group rinsing with acidulated NaF showed little change in oral

137

flora using the methods and the parameters of this study. This finding is in agreement with others. While elevated concentrations of fluoride are bactericidal mouthrinse levels of NaF appear to have little effect on salivary flora.

The lack of effect of both fluoride agents and dental treatment on lactobacilli was not surprising. Neither chlorhexidine nor fluoride has shown potential in supressing this microorganism. Also in the present study, the great variability of lactobacilli counts even in the same individual, limited the meaningfulness of this parameter. Even though salivary lactobacilli recoveries are known to correlate with caries, this organism is no longer considered as important as S. mutans for the initiation of dental caries.

Conclusion

Twenty-two subjects, who were regarded as potentially caries active, rinsed twice a day with either acidulated NaF or SnF_2 mouthrinses, adjusted to 200 ppm F^-. At baseline, and after 1, 3, 6, and 12 months each subject's saliva was analyzed for Total CFU, S. mutans, and lactobacilli.

There was a small (2 times) but significant reduction in Total CFU per ml saliva in both groups after a year. No differences were found in lactobacilli counts between the 2 mouthrinse groups or longitudinally within the groups. Of importance was the apparent selective reduction in S. mutans found in those subjects rinsing with SnF_2. While there was essentially no change in S. mutans in the NaF group, all subjects in the SnF_2 group had large reductions. At the end of 1 year the SnF_2 group had less (26 times) fewer S. mutans compared to baseline. The reduction in S.

mutans levels correlates to the reported lower caries scores in the subjects rinsing with SnF_2.

The selective antimicrobial actions of SnF_2 against S. mutans and the established physicochemical action of SnF_2 with tooth enamel may make this agent superior to other fluoride agents in the treatment of subjects having high caries activity associated with elevated S. mutans levels.

STUDY 4: EFFECT OF SNF$_2$ AND ACIDULATED NAF MOUTHRINSE ON CARIES INCIDENCE

IN ADULTS WITH HIGH NUMBERS OF S. MUTANS AND HIGH CARIES PREVALENCE

INTRODUCTION

It is well documented that topically applied fluorides decrease caries activity, but there has been no strong evidence demonstrating the relative effectiveness of fluoride compounds. Currently, fluoride is believed to be effective, in part, due to its ability to stimulate remineralization. Other physicochemical and antibacterial mechanisms may have important anticaries action as well. The principal fluoride solution used today, NaF, decreases plaque formation when applied at high concentrations, but not at mouthrinse levels. Stannous fluoride (SnF$_2$), however, has been shown to have antibacterial properties at mouthrinse concentrations in vitro and in vivo.

Children with a high caries incidence harbor large numbers of Streptococcus mutans per ml saliva. When the number of S. mutans is decreased, the caries incidence is reduced. Even though it has been suggested that topically applied NaF should be more effective in high risk children, at least one study has shown that NaF has little effect on subjects with high caries activity indicating that factors such as microorganisms and diet cannot be nullified by NaF. SnF$_2$ mouthrinse with its potential antimicrobial activity, however, has not been tested specifically on high risk subjects. Furthermore, while it has been shown that children with high DMFS scores and high numbers of S. mutans are at risk for caries, such studies in adults are largely lacking.

The aims of this study were: 1. to compare the effectiveness of SnF$_2$ and acidulated NaF in adults with high numbers of S. mutans and high caries

140

prevalence and 2. to determine if the number of S. mutans and the caries prevalence can be used to predict the incidence of caries in adults.

MATERIALS AND METHODS

Subjects

The subjects in this study were adults over the age of 18 living in a fluoridated area. They had incipient carious lesions, high numbers of unrestored carious lesions and elevated numbers of S. mutans in their saliva indicating high caries activity. Of the 58 subjects first identified by their caries prevalence in the screening clinic at the University of Connecticut School of Dental Medicine, subsequent microbial screening showed that 38 subjects had greater than 2×10^5 S. mutans per ml saliva. These subjects were regarded as potentially highly caries active . The 37 subjects who consented to the study were ranked by their number of S. mutans and then alternately assigned to a SnF_2 or an acidulated NaF mouthrinse group. During the first year 15 patients withdrew from the study. Of the remaining 22 patients, 9 were considered partially compliant with the mouthrinsing procedures. Partially compliant subjects were those who, by their report, missed more than 4 mouthrinses per month, or who were inconsistent with mouthrinsing. We verified these reports by monthly monitoring of each patient's remaining supply of mouthrinse and by questioning the patients for monthly recall of their fluoride usage.

Treatment

After baseline data were obtained, subjects were instructed to use 10 ml of their respective mouthrinses twice daily for 1 minute per rinse. The SnF_2 rinse (Iradicav[R], Johnson & Johnson Co., East Windsor, NJ) was diluted

with water (1:4) immediately before use to produce a final fluoride concentration of 200 ppm F⁻ and a pH of 3.4. The acidulated NaF mouthrinse (Phos-Flur Oral Rinse^R, Hoyt Laboratories, Needham, MA) was used full strength at a fluoride concentration of 200 ppm F⁻ and a pH of 4.0. One month after the initiation of mouthrinsing, each subject received 3 dental hygiene appointments at one week intervals. The oral hygiene instruction, cleaning, scaling and root planing were performed by one dental hygienist, blind to the subjects' treatment categories. The subjects were also assigned to a dental resident for restoration of teeth with active carious lesions. They were contacted monthly to reinforce oral hygiene, to monitor their fluoride usage and to resupply them with mouthrinse.

Clinical Examinations

Caries recording was performed with the aid of a front surface dental mirror, explorer and posterior bite-wing radiographs. Prior to examination the teeth were cleaned and dried. All decayed, filled and missing surfaces in the permanent dentition were recorded excluding third molars. Incipient smooth surface lesions, not included in the DMFS score, were described by their size. All recordings were performed by the same dentist. The diagnostic error was calculated by duplicate recording performed on 8 subjects, 5 to 14 days after baseline examination. The reproducibility score, i.e. the number of surfaces diagnosed as carious or sound at both examinations, was 93%. At the end of the year the subjects were re-examined for new lesions.

Microbiology

Stimulated saliva from each subject, produced by chewing on a piece of paraffin wax, was collected at the screening and baseline examinations. Each saliva sample was immediately vortexed, diluted in 0.05M phosphate

buffer (pH 7.0) and plated on Mitis-Salivarius agar containing 0.2 units/ml Bacitracin (MSB) by the micromethod of Westergren and Krasse. The agar plates were incubated for 48 hours in a CO_2-enchriched environment (candle jar). The mean count of the two samples was considered the patient's number of S. mutans/ml saliva. Subsequent saliva samples were taken at 1, 3, 6, and 12 months.

Statistical Methods

The differences in caries incidence between the two groups were analyzed non-parametrically by the Wilcoxon two-sample ranks test for unpaired measurements. All tests were performed at the 0.05 level of significance.

RESULTS

The subjects, who were alternately assigned to either the acidulated NaF or SnF_2 groups by their levels of initial S. mutans/ml saliva, had other baseline characteristics that were well distributed. The subjects in the acidulated NaF group presented with means of 7.5 white spot lesions, 15 unrestored carious lesions and a total mean DMF(S) of 63.2. The group asssigned to rinse with SnF_2 had means of 7.8 white spot lesions, 12.7 unrestored carious lesions and a total mean DMF(S) of 76.4 (Tables 14 & 15). Note that partially-compliant and compliant subjects were considered one group in the statistical analyses.

The population of subjects was generally unreliable. Of the 38 subjects who started the study, 22 were in the study after 1 year and, of these, 13 were identified as being completely compliant with the instructions to rinse twice every day over the study year.

During the year, all subjects who rinsed with acidulated NaF developed new carious lesions. The mean numbers of new lesions were 4.3

and 4.5, respectively for the compliant and partially-compliant acidulated NaF rinsing groups. Of the 44 new lesions after 1 year in this group, 10 (23%) were recurrent lesions (Table 15). The caries activity after 1 year in the SnF_2 group was significantly lower. The mean numbers of new lesions for the SnF_2 group were 2.3 and 2.8 respectively for the compliant and partially-compliant groups. Two subjects who rinsed regularly with SnF_2 developed no new lesions, while 1 subject in both the compliant and partially-compliant group developed 5 new lesions. Recurrent decay contributed 28% of the total new lesions in this group (Table 15).

DISCUSSION

Caries prevalence was used as the primary criterion to select adults with high caries risk, since it has been demonstrated that caries incidence correlates to caries prevalence. In this study, however, the numbers of unrestored and incipient carious lesions were considered more important in identifying high risk patients because Klock and Krasse have shown that these parameters are better correlated to caries incidence than missing and filled surfaces. Based on the clinical data, potential subjects were screened for high numbers of salivary S. mutans since, at least in children, high caries prevalence in combination with high numbers of S. mutans correlates better to caries activity than caries prevalence alone. According to the limits set by Zickert et al. subjects with more than 200,000 S. mutans /ml saliva were considered at high risk and accepted for the study.

Because all subjects received fluoride, it is not possible to show the true caries incidence for this type of population. It is reasonable to assume that our selection process for high risk patients was valid since

144

both the incidence and the caries prevalence in the two test groups in this study were considerably higher than Lu et al. reported for adults in a nonfluoridated area of the U.S. (DMFS=38; DMFS=0.69/year). It should be noted, however, that Axelsson and Lindhe reported caries prevalence and caries incidence in a Swedish population similar to those in the present study.

The results showed that rinsing with SnF_2 was more effective than acidulated NaF in highly caries active patients. A possible explanation for this might be the better antimicrobial effect of SnF_2, demonstrated by the lowered S. mutans levels in the SnF_2 group. This is in agreement with a study by Zickert et al. who showed that another antibacterial agent, Chlorhexidine gluconate, reduced both S. mutans and caries incidence in children with high caries activity. Thus, highly caries active patients screened by means of elevated S. mutans may be effectively treated by agents directed against the pathological microorganisms. It should be noted that even though the SnF_2 rinsing reduced the caries incidence more than acidulated NaF, the remaining caries activity was still high. Similar finding have been reported by Seppä et al.

Aside from the number of S. mutans, high caries activity can be the result of low enamel resistance, high sucrose intake and/or low salivary flow. With factors other than elevated pathogenic microorganisms, SnF_2 may not be better than other fluoride compounds at reducing caries activity. The effect of these other factors may explain why highly caries active patients given the same treatments may be affected differently.

We cannot tell if the acidulated NaF treatment and/or oral hygiene appointments had any effect on caries incidence in this population due to the lack of a control population. Both a positive effect and a poor effect

145

for NaF treatment have been reported in highly caries active children. It is obvious, though, that a finding of 4.4 new lesions after 1 year in the acidulated NaF group is extremely high, and consequently the daily acidulated NaF regimen appears to have had only a limited effect.

A surprising finding in this study was that those patients who were identified as strictly adhering to the regimen (rinsing twice a day) did not seem to develop fewer lesions than those who were known to use the mouthrinse less frequently. This finding is in contrast to other studies showing that frequent fluoride exposures are inversely related to development of new lesions. Possibly either our criterion for strict compliance (missing less than 4 rinsings out of 60 per month), or a more than optimium fluoride regimen, could contribute to the lack of difference between the compliant and partially compliant subjects.

The drop-out figure (45%) was very high. No study similar to this one has been reported and perhaps rinsing twice a day is excessive behavioral change for patients who have high caries experience.

In conclusion, this study found that highly caries active patients could be identified by means of caries prevalence and salivary S. mutans levels. All patients continued to be caries active after one year despite the use of two daily fluoride mouthrinses; however, the subjects rinsing with SnF_2 developed approximately half the number of new carious lesions to those subjects rinsing with acidulated NaF.

146

STUDY 5: EFFECT OF SNF$_2$ AND ACIDULATED NAF MOUTHRINSES
ON PLAQUE AND GINGIVITIS IN ADULTS WITH HIGH CARIES PREVALENCE

INTRODUCTION

Efforts to prevent or treat periodontal diseases are aimed at the control of plaque, either through mechanical or chemical means. Based on numerous short and long term studies, the antiseptic chlorhexidine is now the most used adjunct in treating these diseases. Short term studies also have demonstrated SnF2 to be effective in plaque reduction when used as a mouthrinse twice daily. Only one study has examined the use of SnF2 on a long term basis. In this study, school children rinsing once a day for 4 months with SnF2 had lower plaque scores than those rinsing with NaF, but no difference was found in gingivitis scores between the two groups.

As part of a comprehensive study on adults with rampant caries adjunctively rinsing with either SnF2 or NaF, we examined their plaque and gingivitis levels longitudinally. This report discribes the differences between the two groups in the periodontally related parameters after 1 year.

147

MATERIAL AND METHODS

The subjects for this study were selected by their high caries prevalence, and by their high numbers of salivary *S. mutans*. Of the initial 37 subjects, 22 remained in the study after 1 year. The subjects were further catagorized as compliant or partially compliant. Partial compliance was defined as those who, by their own report, missed more than 4 mouthrinses per month, or who were inconsistant with their mouthrinsing. These reportrs were verified by monitoring the patients remainig supply of mouthrinse and by monthly questioning of the patients regarding their fluoride usage.

Following baseline examination, subjects were instructed to use 10 ml of their mouthrince twice daily for one minute per rinse. The SnF_2 rinse (Iradicav, Johnson and Johnson Co., East Windsor, N.J.) was diluted with water (1 part SnF_2: 4 parts water) immediately before use to produced a final fluoride concentration of 200 ppm and a pH of 3.4. The acidulated NaF mouthrinse (Phos-Flur Oral Rinse, Hoyt Laboratories, Needham, Mass.) was used at full strength at a concentration of 200 ppm fluoride and a pH of 4.0. One month after the initiation of mouthrinsing, each subject had 3 dental hygience visits at one week intervals. Oral hygience instruction, prophylaxis, and scaling and root planning were performed by one dentral hygenist, blind to the patients treatment catagory. The subjects also received complete restorative treatment by dental residents. In order to reinforce oral hygience, monitor fluoride usage and resupply the subjects with fluoride mouthrinse, they were contacted monthly.

The baseline and 1, 3, 6, and 1 year subsequent examinations included

148

gingival index and plaque index which were recorded by one examiner, blind as to the subjects groupings. The GI and PI1 data was reduced to frequency of scores and means for each subject. The frequency scores were then converted to "% of sites with plaque" (ie. PI1 score of 1,2,or3), and to "% of sites with bleeding" (ie. GI score of 2 or 3). Individual percentages were then averaged for each group. The scores for each subject were also converted to mean GI and PI1 and then analyzed as parametric data using analysis of variance for repeated measures. This test enabled longitudinal as well as cross sectional evaluation of each group.

RESULTS

The subjects in this investigation represented essentially a poorly compliant population. Sixteen subjects dropped out in the first year; and of the remaining 22, only 13 were considered compliant in their use of the mouthrinse.

After one year, both the SnF2 and the NaF "Total" (compliant and non-compliant subjects) groups demonstrated a significant decrease in plaque (mean differences of 66% and 62%, respectively. However, no significant difference was found in plaque scores betweeen the SnF2 and NaF groups (Table 16).

Gingival inflammation was significantly reduced in the Total SnF2 group, both longitudially from baseline (23%), and cross sectionally compared to the Total NaF group (17%). Statistical differences in the GI reduction between groups is seen in the Total and Compliant groupings, but not in the Non-compliant subjects when analyzed separately (Table 17).

A further analysis was performed to correlate individuals mean plaque score to their mean gingival score. Plaque scores were positively correlated to gingival scores in those subjects rinsing with NaF (r =.83); however, in the SnF2 group the subjects plaque scores had a significant inverse relationship to the gingival scores (r = -.57).

150

DISCUSSION

The reduction in plaque scores from baseline to 1 year in both the SnF2 and NaF rinsing groups was expected sine all subjects had thorough instruction and reinforcement in oral hygiene. This significant improvement in the subjects oral hygiene and the eventual low plaque scores probably hinders the possibility of observing differences in plaque scores between groups. Furthermore, several studies have described a increased in non-bacterial pellicle on tooth surfaces of subjects rinsing with SnF2. Since pellicle is not readily distinguishable from plaque with clinical indices, this tooth deposit would also confound plaque scores. Evidence that subjects in the SnF2 group had non-bacterial deposits on their teeth is suggested by the negative correlation between the plaque scores and the gingival scores in the SnF2 group.

This study did find that SnF2 was an adjunct in decreasing gingival inflamation. The lower frequency of bleeding sites and the corresponding lower mean GI scores in the SnF2 group compared to the NaF group demonstrates that rinsing with SnF2 favorably affected gingival health. Since we have also noted decreased caries, and greatly reduced $S.$ mutans in these same subjects rinsing with SnF2 compared to NaF, SnF2 may have advantages at least in subjects with dental disease. This noted effect of SnF2 on gingivitis levels differs from the only other long term examining this parameter. Perhaps our population of adults with existing gingivitis, or the fact that our subjects rinsed twice a day, seven days a week could account for the differences.

Theoritically SnF2 has the potential of affecting gingival health.

151

Studies in vivo have shown that SnF2 irrigated into periodontal pockets dramatically reduces the number of presumptive periodontopathic microorganisms and gingival bleeding, and in vitro studies have shown that SnF2 is more effective than other fluoride compounds in inhibiting growth and viablity of B. melogenicus, and Actinomyces species.

From several short term studies showing antiplaque effects of SnF2 and the present study showing a small but significant affects on gingivitis, frequent rinsing with SnF2 may have greater effect than other fluoride rinses in treatment of subjects with high caries activity and periodontal disease. However, as we observed in this study it may be difficult for the average patient to follow a regime of twice a day rinsing over an extended period. Perhaps other delivery systems or regimens that don't require as much patient cooperation are necessary as a large scale public health measure.

STUDY 6: SAFTEY AND ANTIBACTERIAL PROPERTIES OF

CONTROLLED RELEASE SNF$_2$

INTRODUCTION

Sustained release delivery of drugs has several advantages: (1) It enable lower dosage of drugs because the agent is released near the intended site of action, (2) It overcomes problems of side effects because of the lower therapeutic levels; (3) It reduces need for patient envolvement; and (4) It eliminates the need for frequent drug administration.

In dentistry sustained release systems have been explored for delivery of steroids, anti-fungal drugs; antibacterials ; and fluorides for the control of dental caries and remineralization. To date the largest a clinical study has been performed with a trilaminate methacrylate sodium fluoride-releasing device cemented to the buccal surfaces of teeth of 11 subjects. The intraoral device was found to elevate the levels of fluoride in plaque, saliva and urine, but had no effect on plaque or gingival parameters.

Fluoride ions may act as a therapeutic agent by altering bacterial metabolism as well a reacting physicochemically with enamel to reduce enamel solubility or remineralize initial caries. Stannous fluoride has been shown to have a greater effect on bacterial metabolism than other fluoride compounds. Pilot studies with stannous fluoride incorporated in

polycarboxylate cement and used as a temporary Intercoronal restoration have been performed to test its potential as an antiplaque device. These studies, performed _in_ _vitro_ and in one subject, demonstrated that the SnF_2-polycarboxylate cement had sufficient compressive strength, released of fluoride at therapeutic levels, had acceptable clinical properties, and caused a visual change in plaque formation .

Based on these favorable initial experiments, the present clinical study was performed to further evaluate the saftey and efficacy of the SnF_2-polycarboxylate Intercoronal restoration in two human clinical trials.

MATERIALS AND METHODS

Safety and Fluoride Release

Eight subjects were used to test: 1. the integrety of the SnF_2 polycarboxylate cement as an intracoronal restoration; 2. the potential side effects; 3. the oral release of fluoride from the cement, and 4. the charge of fluoride levels in the urine. Subjects consisted of dental students and dental assistants who consented to the study and had a defective restoration in a molar tooth requiring at least a two surface restoration. The trial period, when the slow release restoration was in place, lasted 34 days.

A week prior to the trial and during the trial period, all subjects were given fluoride free toothpaste and instructed to use only this toothpaste until the experiment was over.

The SnF_2-polycarboxylate cement was prepared by combining pulverized SnF_2 with polycarboxylate powder (Durelon, Premier) in a ratio of 1:1 (W/W). The fluoride crystals were pulverized to a fine powder by triturating the compound in a dental amalgamator (Wiggle-bug, LPGO, Cresent Dental) for 1 minute at maximum velocity.

The test tooth in each subject was prepared conventionally for an intracoronal restoration. Orthodontic bands were then fitted around the test tooth. (The bands were used to prevent tooth drift in case the temporary restoration was lost prematurely.) The SnF_2-polycarboxylate powder was mixed with the polycarboxylate liquid according to manufacturers instructions and used to cement the orthodontic band around the tooth as well as restoring the tooth. Approximately 300mg SnF_2 (72mg F^-) was used in each restoration. The teeth were kept dry throughout all procedures with the aid of rubber dam isolation. After the cement had hardened, the

155

rubber dam was removed and occlusion of the restoration was adjusted. Besides giving the subjects instructions about giving saliva and urine samples, the subjects maintained normal oral hygiene and activity. The temporary restorations were photographed, approximately 1:1, at the time of placement and again at the end of the trial period. At the end of the trial, the slow release restorations were replaced with either amalgam or gold restorations.

Twice prior to the trial period and twice weekly during the trial period, salivary and urinary samples were collected between 8 and 10 a.m. Salivary samples consisted of whole saliva, stimulated by having subjects chew on parafin. Saliva and urine samples were diluted 1:1 with ionic strength buffer (TISAB with CDTA). The fluoride ion concentrations of the samples were then determined using a combination fluoride electrode (Orion, model 96-09) connected to a digital readout electrometer. Millivolt readings of the samples were compared to those of NaF standards.

Efficacy

Fourteen subjects were alternately assigned into 2 groups so that half had a controlled release SnF_2 restoration placed in a molar and half had a placebo restoration (IRM). As in the previous trial the subjects were dental students or assistants who required two or more surface restoration in a permanent molar.

A two week initial preparation period, in which all subjects were scaled, polished and given detailed instructions in effective plaque control, preceded the experimental period. On day one of the experimental period a temporary restoration of either 50% SnF_2 in polycarboxylate cement or IRM was placed within a cavity preparation of a tooth of the appropriate subject. The procedure was identical to the previous trial except that orthodontic bands were not used this time around the restoration. Subjects

156

were then instructed to abstain from all forms of active oral hygiene for the next 14 days. Following the 14 days trial period, permanent restorations were placed in the test teeth, oral hygiene was reinstituted, and each subject received a profesional tooth cleaning. Subjects continued to be followed for 2 weeks after the experimental period (Figure 6).

Microbiologic baseline samples were taken twice before the trial (one week prio and immediately before the temporary restoration was placed). Microbiologic samples from each subject were again taken on day 7 and 14 of the trial and twice during the post-trial period. The microbiologic procedures consisted of enumerating total colony forming units, _S. sanguis_, and _S. mutans_ from salivary samples. Stimulated saliva from each subject was collected as previously described in the morning for each designated period. One ml of saliva from each subject was diluted with 3 ml of reduced transport fluid without ethylenediamine tetraacetate, sonicated for 10 sec. at output setting 4 (Bronson, B15, with microtip), and then serially diluted. Samples were plated onto enriched trypticase soy blood agar, MM10 with 20% sucrose and HLR with 20% sucrose and 0.2 units bacitracin. Special sectors containing 20 to 50 colonies were quantitated for total colony forming into units, _S. sanguis_ and _S. mutans_, respectively. _S. sanguis_ and _S. mutans_ were identified by morphologic criteria. Questionable colonies of _S. mutans_ were subjected to biochemical analysis. The mean of 2 samples for each time interval was used to distrbute each subject into ranges with regard to total colony forming units, _S. sanguis_ and _S. mutans_.

Clinical measurements of gingivitis-G.I. and plaque-PlI were taken on each subject immediately prior to placement of the temporary restoration and measurements were repeated on days 7, 14, and 28. The mean frequency

of scores "0" and "2" for each subject were used to determine the group mean scores of "0" and "2" at each scoring period.

RESULTS

Safety and Fluoride Release

Of the 8 subjects who had the SnF_2-polycarboxylats temporary restorations placed in a molar tooth, 7 completed the 34 day trial. One subject was discontinued after 5 days because the proximal portion of the restoration broke. All the subjects on the first day experienced pain and gingival irritation where the orthodontic band and SnF_2- polycarboxylate cement that was used to cement the orthodontic band touched the gingiva. Because of the irritation, the orthodontic band and cement that held the band was removed from all subjects after the second day.

The baseline salivary fluoride levels for all subjects was less than 0.5 $ppmF^-$. The salivary fluoride levels increased to a mean of 1.5 ppm on the second day after the restoration was in place. The salivary fluoride levels sharply declined on day 6 to a mean of 0.5 ppm and then gradually decreased during the rest of the trial. Detectable levels of fluoride above baseline were still found at 4 weeks. Over the course of the 34 day trial the mean salivary fluoride level was 0.3 ppmF (Figure 7).

Baseline urinary fluoride levels were approximately 0.7 ppmF. On the second day of the trial the mean urinary fluoride level of the subjects was 2.2 ppm. Urinary fluoride levels were only slightly above baseline after day 6 (Figure 8).

Except for a delay in the setting time (approximately 15 minutes), the SnF$_2$-polycarboxylate combination was similar to that of unaltered polycarboxylate cement. Excluding the one broken restoration, the integrity of the filling material was excellent during the trial period. There was no noticeable wear of the restoration on the margins or on the proximal contact. All restorations, however, had a change in color from white to dark grey (Figure 9).

Efficacy

Subjects receiving the SnF$_2$-polycarboxylate cement and whose cavity design extended subgingivally again experienced pain and gingival irritation lasting 2-3 days. Gingival sloughing of the sulcular epithelium appeared to have occurred in 2 subjects who had preparations extending several millimeters subgingivally.

Categorization of subjects by the number of total colony forming units/ml saliva revealed a shift to increased number of bacteria in those subjects receiving the control cement during the experimental period. No increase in total CFU was observed in those subjects having the SnF$_2$ restoration (Table 18). Subjects distributed by S. mutans recoveries at the different intervals revealed that during the experimental period there was a shift upward in S. mutans in those subjects having the placebo while there was a shift downward in those subjects having the SnF$_2$ restoration (Table 19). As with total CFU, the S. sanguis counts increased during the experimental period and decreased in the post-experimental period (Table 20). However, no difference between the SnF$_2$-polycarboxylate group and the placebo group could be noted at any time interval.

Plaque scores of all the subjects were excellent at baseline as shown by the high number of "0's" and the low number of "2's". After 7 days of no oral hygiene in the experimental period, both groups had a similar high

number of "2" scores. At 14 days, the frequency of score "2" was 16% less
in the SnF$_2$ group. At the end of the 14 day post-trial period, the large
number of "0" scores indicated reinstitution of excellent oral hygiene
practices, but the placebo group showed a 10% higher number of "0" scores
(Table 21).

The gingival health of the subjects was also excellent as shown by the
high frequency of "0" GI scores in both groups. Little change in gingival
health was noted until the end of the trial period (2 weeks without oral
hygiene). No difference, however, was detected in gingivitis levels at
this time. The post-trial GI scores showed that all subjects' gingival
health returned to baseline levels (Table 22).

DISCUSSION

The present clinical trials were designed to examine the safety and
efficacy of a controlled release delivery system of SnF$_2$ in a small number
of human subjects. Although the study designs do not permit statistical
tests of significance, certain trends are apparent.

The compatibility of large quantities of SnF$_2$ in polycarboxylate
cement, as shown in a previous pilot study is apparent. The SnF$_2$
restorations showed no signs of wear or loss of integrity in both the
trials. The release of SnF$_2$ from polycarboxylate cement as measured by the
salivary fluoride levels over a 34 day period, was similar to release
patterns of other drugs from controlled release devices . There was an
initial large release of fluoride in the first days followed by a an linear
decline in salivary fluoride levels over month period. The mean fluoride
levels over the month period of 0.3 ppm F may have been less than optimal.
This release rate, however, may be realistic relative to having only one

restoration in the mouth of each subject. The finding of only minor increases in urinary fluoride levels further substantiates the systemic safety of this release system.

The side effect of gingival irritation and consequent patient discomfort where the restoration touched gingival tissues can not be ignored. Both SnF_2 and acidulated NaF have been reported to cause irritation, of crevicular epithelium especially in the presence of gingival inflamation. In this delivery system, where gingival tissues are irritated due to operative procedures, inflamation of the gingiva surrounding the tooth receiving the temporary restoration can not be avoided. However, in the future, avoiding soft tissue contact of the SnF_2-polycarboxylate restoration may be possible by placing this restoration only in situations where it does not touch the gingiva, (i.e. Class I restorations), or by protecting the gingiva with an orthodontic band cemented around the tooth with a non-fluoride containing cement prior to placement of the controlled release restoration.

Some effect on both the quantity and proportion of microorganisms was noted in those subjects who had a SnF_2-polycarboxylate restorations in place. While there was an increase of recovery of total colony forming units from salivary in the placebo group during the experiemental period, probably due to suspension of oral hygiene in this period, a decrease in total bacteria was noted in the SnF_2 group. This decrease in salivary microorganisms may be selective since S. sanguis recoveries showed no difference between group but S. mutans recoveries appeared less in those subjects having the SnF_2 restoration. This selectivity of SnF_2 against S. mutans has been previously observed.

The effect of the SnF_2 delivery system against plaque and gingivitis was not immpressive. Only at one data point, the frequency Pl' score, at

day 14, showed a reduction of 16% for those subjects who had the SnF_2 controlled release devices in place. We had little expectation of finding differences between groups with regard to these parameters. The large pellicle deposits produced by SnF_2 interfere with traditional plaque scoring methods. Other measurement systems which take this problem into account should be used when visualizing plaque in a study using SnF_2. Gingivitis scores were not different between the SnF_2 group and the placebo group. However, the study period of only 2 weeks does not permit enough time for gingivitis to develop in experimental gingivitis model. Longer term studies using SnF_2 at higher concentrations in animals and humans have shown reduced gingivites due to SnF_2.

These present studies must be compared to the results found with a recently reported study with controlled release NaF. In that study 2 membrane controlled release NaF. In that study, two membrane controlled devices containing 42 mg each of NaF (total of 38 mg F^-/subject) were cemented to first molar of 11 subjects. Side effects in that study were that 2 devices fell off and several subjects reported that the device caused irritation to soft tissues. The membrane controlled devices did increase fluoride levels in saliva and plaque, but no changes could be found in clinical plaque, gingivitis or microbial parameters.

Obviously, devices for the controlled release of fluoride need more development and clinical trials before they can be accepted as a therapeutic approach on a population basis. Further clinical trials that have reduced tissue irritation, increase the amount and duration fo fluoride release and greater bacterial effect need to be achieved. Perhaps use of SnF_2 in these release systems, because of its apparent selectivity against S. mutans and employment of an intercoronal delivery system because

the quantity of fluoride compounds can be increased in subjects requiring

restorations of multiple teeth, should be further explored.

Agent	%	Cation (ppm)	Anion (ppm)	pH's
H_2O	---	---	---	2.5, 6.0*
NaF	0.055	303	250	2.5, 3.0, 5.5*, 6.0, 7.0
SnF_2	0.103	783	250	2.0, 2.5, 3.0, 3.5*, 4.0, 5.0, 6.0, 7.0
$SnCl_2$	0.124	783	463**	2.5*, 7.0
SnF_4	0.064	390	250	2.3
ZnF_4	0.068	428	250	5.2*
CuF_2	0.067	418	250	3.0, 6.0
PbF_2	0.065	545	100***	3.0, 6.0

*unadjusted pH
**$SnCl_2$ prepared to be cationicly equal to that of SnF_2.
***PbF_2 not soluble at 250 ppm F.

Table 1: List of agents tested against S. mutans. Percentage of compounds prepared to give same anion concentration.

Agent	Agent pH	Acid Production (Δ pH)	Plaque Score[a]	Plaque Weight (mg)	Metal/mg Plaque (μg)
NaF	5.5	2.7	3	9.8 ± 0.6	N.D.
ZnF_2	5.2	2.8	3	10.0 ± 0.5	0.05 ± 0.01
SnF_4	2.3	2.6	3	10.9 ± 0.2	8.9 ± 2.0
SnF_2	3.5	0.4	<1	1.3 ± 0.4	39.1 ± 1.4

[a] Scored by McCabe method

N.D. = Non-detected

N=3; $\bar{x}$ ± S.D.

Table 2: Effect that twice daily exposure of listed fluoride compounds (250 ppm F⁻) had on several growth parameters and metal uptake of S. mutans NCTC 10449.

Agent	Agent pH (adjusted)	Acid Production (Δ pH)	Plaque Score[a]	Plaque Weight (mg)	Metal/mg Plaque (μg)
H_2O	2.5	2.8	4	11.0 ± 0.2	N.D.
	7.0	2.7	4	10.7 ± 0.5	N.D.
NaF	2.5	2.5	4	12.5 ± 0.5	N.D.
	7.0	2.7	4	11.2 ± 0.9	N.D.
$SnCl_2$	2.5	2.7	4	12.3 ± 0.6	1.4 ± 0.6
	7.0	2.7	4	11.1 ± 0.7	0.3 ± 0.1
SnF_2	2.5	1.7	2	7.2 ± 2.0	13.6 ± 4.1
	7.0	2.6	4	13.0 ± 0.5	0.5 ± 0.1

a Scored by McCabe method.

N.D. = Non-detected

N=3; $\bar{x}$ ± S.D.

Table 3: Effect that twice daily exposure of fluoride compounds (250 ppm F⁻) or controls adjusted to low or neutral pH, had on S. mutans NCTC 10449 growth and metal uptake.

Agent	Agent pH (adjusted)	Acid Production (Δ pH)	Plaque Score[α]	Plaque Weight (mg)	Metal/mg Plaque (μg)
	2.0	0.2	1	1.8 ± 0.1	λ
	3.0	0.2	1	2.4 ± 0.5	42.9 ± 7.1
SnF_2	4.0	0.5	1	2.6 ± 0.5	36.9 ± 3.6
	5.0	1.0	3	5.7 ± 0.4	20.1 ± 0.5
	6.0	1.6	4	5.9 ± 0.8	3.6 ± 0.7

α Scored by McCabe method.

λ Laboratory accident.

$N = 3$; $\bar{x}$ ± S.D.

Table 4: Effect that twice daily exposure of SnF_2 (250 ppm F^-) at various pH's had on S. mutans NCTC 10449 growth and metal uptake.

Agent	Agent pH (adjusted)	Acid Production (Δ pH)	Plaque Score[a]	Plaque Weight (mg)	Metal/mg Plaque (μg)
NaF	3.0	1.9	3	5.7 ± 0.2	N.D.
	6.0	2.3	3	4.6 ± 1.0	N.D.
PbF$_2$	3.0	1.9	3	6.7 ± 0.3	3.3 ± 0.5
	6.0	2.0	3	5.5 ± 0.1	2.4 ± 0.7

a Scored by McCabe method.

N.D. = Non-detected.

N = 3; $\bar{x}$ ± S.D.

Table 5: Effect that twice daily exposure of NaF (250 ppm F$^-$) or PbF$_2$ (100 ppm F$^-$) had on <u>S</u>. <u>mutans</u> NCTC 10449 growth and metal uptake.

Agent	Agent pH (adjusted)	Acid Production (Δ pH)	Plaque Score[α]	Plaque Weight (mg)	Metal/mg Plaque (μg)
NaF	3.0	2.39	5	13.3 ± 0.9	N.D.
	6.0	2.33	5	12.4 ± 1.9	N.D.
CuF_2	3.0	2.42	5	12.7 ± 1.6	Trace
	6.0	2.48	5	13.0 ± 0.8	Trace

α Scored by McCabe method.

N.D. = Non-detected.

N = 3; $\bar{x}$ ± S.D.

Table 6: Effect that twice daily exposure of NaF or CuF_4 (250 ppm F⁻) had on S. mutans NCTC 10449 growth and metal uptake.

Agent	Agent pH	Acid Production (Δ pH)	Plaque Score$^\alpha$	Plaque Weight (mg)	Metal/mg Plaque (μg)
H_2O	6.0	2.2	5	8.3 ± 1.0	N.D.
SnF_2	3.5	1.3	3	12.2 ± 0.7	9.7 ± 2.5

α Scored by McCabe method.

N.D. = Non-detected

N=4; $\bar{x}$ ± S.D.

Table 7: Effect that twice daily exposure of SnF_2 (250 ppm F^-) had on preformed S. mutans NCTC 10449 plaque.

| Tx | n | CARIES SCORE | | | | MICROBIAL RECOVERY |
		ENAMEL	Red.	DENTINAL	Red.	$X10^6$
H_2O	13	6.54 ± 1.80†	—	3.23 ± 2.45	—	37.6 ± 36.0
NaF	14	3.85 ± 1.51*	41%	0.57 ± 0.94*	82%	19.9 ± 21.4
SnF$_2$	13	3.69 ± 2.10*	44%	0.46 ± 0.78*	86%	27.6 ± 32.5

† $\bar{x}$ ± S.D.
* Significantly different from deionized H_2O group, $p \leqslant .05$

Table 8

Caries Scores and S. Mutans Recovery in Hamsters Drinking Deionized H_2O, NaF, or SnF$_2$ (5 ppm F⁻) for 64 Days.

Agent	n	Baseline*	1 Month	3 Months	6 Months	12 Months
Complete Compliance						
NaF	6	28.8 ± 29.5	10.6 ± 3.1	20.5 ± 23.9	11.6 ± 7.5	11.3 ± 5.7
SnF_2	7	20.3 ± 12.8	17.4 ± 18.5	5.8 ± 3.8	15.8 ± 17.1	6.6 ± 5.7
Differences†			---	---	---	---
Partial Compliance						
NaF	4	21.7 ± 10.5	18.8 ± 9.4	22.2 ± 14.2	29.1 ± 35.4	14.0 ± 10.3
SnF_2	5	19.4 ± 9.5	16.5 ± 9.2	18.5 ± 27.0	10.2 ± 7.1	15.9 ± 23.8
Differences†			---	---	---	---
Total Group						
NaF	10	26.0 ± 23.1	13.8 ± 7.2	21.2 ± 19.6	18.6 ± 23.0	12.5 ± 7.6
SnF_2	12	20.0 ± 11.1	17.0 ± 14.7	12.0 ± 18.7	13.5 ± 13.6	10.5 ± 15.7
Differences†			---	---	---	---

* $\bar{x}$ ± S.D.

† Non-significant

TABLE 9

Number of Total CFU ($\times 10^7$) in different groups at baseline and after 1, 3, 6, and 12 months.

Agent	n	Baseline*	1 Month	3 Months	6 Months	12 Months
Complete Compliance						
NaF	6	4.2 ± 4.0	5.7 ± 6.2	6.6 ± 9.5	8.2 ± 9.3	3.9 ± 4.9
SnF$_2$	7	3.1 ± 1.5	0.1 ± 0.1	0.02 ± 0.03	0.4 ± 0.5	0.3 ± 0.3
Differences[†]			57x	366x	---	---
Partial Compliance						
NaF	4	2.3 ± 1.6	3.8 ± 2.8	2.9 ± 1.0	4.3 ± 2.4	4.5 ± 4.1
SnF$_2$	5	5.7 ± 3.3	1.2 ± 2.2	1.4 ± 1.2	4.8 ± 4.1	0.04 ± 0.05
Differences[†]			---	---	---	111x
Total Group						
NaF	10	3.4 ± 3.3	5.0 ± 2.2	5.1 ± 7.4	6.6 ± 7.3	4.1 ± 4.4
SnF$_2$	12	4.2 ± 2.7	0.6 ± 1.4	0.7 ± 1.1	2.0 ± 3.2	0.2 ± 0.3
Differences[†]			9x	8x	---	26x

* $\bar{x}$ ± S.D.
[†] Differences shown are significant, $p < .1$ by analysis of variance

TABLE 10

Number of S. mutans (x10^6) in different
groups at baseline and after 1, 3, 6,
and 12 months.

S. mutans/ml saliva	Baseline		1 Year*	
	NaF	SnF₂	NaF	SnF₂
> 5 million	3	4	4	---
3-5 million	2	2	1	---
1-3 million	3	5	2	---
200,000-1 million	2	1	2	2
< 200,000	---	---	1	10

*Differs significantly, p< .05 by 2-sample ranks test

TABLE 11

Ranking of all subjects within treatment groups by S. mutans levels at baseline and after 1 year.

174

	Agent	n	Baseline*	1 Month	3 Months	6 Months	12 Months
Complete Compliance	NaF	6	1.0 ± 1.7	0.7 ± 0.8	3.0 ± 5.4	1.2 ± 2.8	1.6 ± 3.7
	SnF$_2$	7	1.3 ± 1.5	1.8 ± 3.7	0.5 ± 0.8	0.7 ± 1.1	1.8 ± 3.2
Differences†				---	---	---	---
Partial Compliance	NaF	4	0.5 ± 0.5	2.1 ± 3.9	1.3 ± 1.2	0.7 ± 0.8	1.5 ± 2.8
	SnF$_2$	5	0.5 ± 0.6	0.6 ± 0.9	1.4 ± 2.3	2.7 ± 3.4	0.8 ± 1.8
Differences†				---	---	---	---
Total Group	NaF	10	0.8 ± 1.3	1.3 ± 2.4	2.3 ± 4.2	1.0 ± 2.1	1.5 ± 3.2
	SnF$_2$	12	1.0 ± 1.3	1.3 ± 3.0	0.9 ± 1.6	1.5 ± 2.4	1.4 ± 2.6
Differences†				---	---	---	---

* $\bar{x}$ ± S.D.
† Non-significant

TABLE 1.

Number of lactobacilli (x10⁶) in different groups
at baseline and after 1, 3, 6, and 12 months

175

Reductions[*]

Microorganism	Agent	n	Times	Percent
Total CFU	NaF	10	2.1x	52%
	SnF$_2$	12	1.9x	48%
S. *mutans*	NaF	10	—	—
	SnF$_2$	12	21x	95%
Lactobacilli	NaF	10	—	—
	SnF$_2$	12	—	—

[*]Reductions shown are significant, p < .05, by t-test

TABLE 13

Reductions in all subjects' salivary micro-organisms at 1 year.

176

Complete Compliance:

Subject	Age	Initial White Spots	D	M	F	S	Primary Caries	Recurrent Caries	Root Caries	Σ	Attac Rate
104	33	8	13	24	31	68	1	--	--	1	1.0
112	26	6	7	4	0	11	7	--	--	7	5.6
305	29	4	19	25	6	50	4	--	--	4	4.1
402	18	7	11	0	56	67	2	3	--	5	3.9
503	37	10	21	29	40	90	2	--	--	2	2.0
504	30	8	12	0	85	97	3	4	--	7	5.5
	$\bar{x}=27.6$	$\bar{x}=7.5$	$\bar{x}=15.0$	$\bar{x}=15.1$		$\bar{x}=63.2$			Subtotal	$\bar{x}=4.3$	$\bar{x}=3.7$

Partial Compliance:

Subject	Age	Initial White Spots	D	M	F	S	Primary Caries	Recurrent Caries	Root Caries	Σ	Attac Rate
203	18	15	24	15	9	48	7	--	--	7	6.2
316	34	3	11	50	4	65	--	1	--	1	1.3
602	33	7	24	4	62	90	1	2	1	4	3.2
603	18	7	8	0	38	46	6	--	--	6	4.7
						$\bar{x}=63.2$			Subtotal	$\bar{x}=4.5$	$\bar{x}=3.9$
									Total	$\bar{x}=4.4$	$\bar{x}=3.8$

Baseline — **New Lesions After 1 Year**

Table 14

Caries prevalence at baseline and caries incidence after 1 year in the acidulated NaF group.

177

Complete
Compliance:

| | | Baseline | | | | New Lesions After 1 Year | | | | |
| | | Initial | | | | Primary | Recurrent | Root | | Attac |
Subject	Age	White Spots	D	M	F (S)	Caries	Caries	Caries	Σ	Rate
102	27	11	5	0	28 = 33	2	--	--	2	2.0
100	69	5	4	35	34 = 73	2	--	1	3	3.2
107	53	2	24	22	29 = 75	2	--	--	2	1.9
114	36	12	13	15	32 = 60	--	--	--	0	0
118	22	14	7	0	69 = 76	--	5	--	5	3.9
701	17	12	36	39	10 = 85	--	--	--	0	0
704	19	12	25	14	51 = 90	4	--	--	4	3.5
	x̄ = 33.8	x̄ = 7.8	x̄ = 12.7	x̄ = 21.8	x̄ = 76.4			Subtotal	x̄ = 2.3	x̄ = 2.1

Partial
Compliance:

Subject	Age	White Spots	D	M	F (S)	Primary	Recurrent	Root	Σ	Rate
103	30	6	6	15	59 = 80	5	--	--	5	4.4
303	28	5	2	15	86 = 103	1	--	--	2	1.8
307	28	2	16	35	40 = 91	1	1	--	2	2.2
401	42	12	5	42	31 = 78	--	1	--	1	1.2
404	34	1	9	30	34 = 73	3	1	--	4	4.1
								Subtotal	x̄ = 2.8	x̄ = 2.7
								Total	x̄ = 2.5*	x̄ = 2.4*

*Statistically different from NaF group.

Table 15

Caries prevalence at baseline and caries incidence after 1 year in the SnF$_2$ group.

178

	Baseline	1 Month	3 Months	6 Months	12 Months
NaF					
Compliant (6)	0.7 ± .29*	0.5 ± .34	0.4 ± .16	0.4 ± .34	0.4 ± .22
Non-Compliant (4)	1.0 ± .78	0.8 ± .82	0.3 ± .27	0.4 ± .24	0.3 ± .08
Total (10)	0.8 ± .53	0.6 ± .55	0.4 ± .21	0.4 ± .29	0.3 ± .17
SnF$_2$					
Compliant (7)	0.5 ± .32	0.3 ± .36	0.3 ± .29	0.3 ± .23	0.2 ± .28
Non-Compliant (5)	0.8 ± .61	0.7 ± .47	0.2 ± .13	0.5 ± .18	0.3 ± .19
Total (12)	0.6 ± .47	0.5 ± .43	0.3 ± .23	0.4 ± .22	0.2 ± .24

*$\bar{x}$ ± S.D.

Table 16

Mean Plaque Score (PI1) for Compliant, Non-Compliant, and Total Subjects at Each Examination.

	Baseline	1 Month	3 Months	6 Months	12 Months
NaF					
Compliant (6)	1.3 ± .24*	1.1 ± .13	1.2 ± .19	1.3 ± .19	1.2 ± .14
Non-Compliant (4)	1.3 ± .35	1.3 ± .36	1.1 ± .03	1.1 ± .03	1.2 ± .09
Total (10)	1.3 ± .27	1.2 ± .24	1.1 ± .16	1.2 ± .17	1.2 ± .12
SnF$_2$					
Compliant (7)	1.3 ± .21	1.2 ± .14	1.0 ± .09	1.1 ± .07	1.0 ± .12+
Non-Compliant (5)	1.4 ± .26	1.3 ± .26	1.0 ± .13	1.2 ± .14	1.1 ± .07
Total (12)	1.3 ± .22	1.3 ± .20	1.0 ± .10	1.2 ± .12	1.0 ± .11+

*$\bar{x}$ ± S.D.

+Significant differences, $p < .05$ between treatment groups over time.

Table 17

Mean Gingival Scores (G.I.) for Compliant, Non-Compliant, and Total Subjects at Each Examination.

	Baseline Period		Experimental Period (no oral hygiene)		Post-Experimental Period	
	Control	SnF$_2$	Control	SnF$_2$	Control	SnF$_2$
10^9-10^{10}	0	0	2	1	0	0
10^8-10^9	5	5	5	3	3	3
10^7-10^8	2	2	0	3	4	4

Table 18 Number of subjects ordered by total salivary colony forming units at baseline, experimental and post-experimental periods. The mean of 2 samples was used to categorize each subject at each period.

	Baseline Period		Experimental Period (no oral hygiene)		Post-Experimental Period	
	Control	SnF_2	Control	SnF_2	Control	SnF_2
$>10^5$	2	3	3	0	2	2
10^4-10^5	2	2	3	4	3	3
10^3-10^4	1	0	0	1	1	0
$<10^3$	2	2	1	2	1	2

Table 19 Number of subjects ordered by salivary S. mutans counts at baseline, experimental, and post-experimental periods. The mean of 2 samples was used to categorize each subject at each period.

	Baseline Period		Experimental Period (no oral hygiene)		Post-Experimental Period	
	Control	SnF_2	Control	SnF_2	Control	SnF_2
10^8-10^9	0	0	0	0	0	1
10^7-10^8	2	3	7	6	4	3
10^6-10^7	5	4	0	1	3	3

Table 20 Number of subjects ordered by salivary S. sanguis counts at baseline, experimental 1 and post- experimental periods. The mean of 2 samples was used to categorize each subject at each period

183

PL 1

	% Frequency Score 0		% Frequency Score 2	
	Placebo	SnF_2	Placebo	SnF_2
Baseline	89	82	3	0
Day 7	3	4	72	75
Day 14	1	2	79	66
Post-Trial	91	81	1	1

Table 21   Mean frequency per subject of   scores 0 and 2 in subjects having slow release SnF2 restorations (N=7) or a placebo restoration (N=7) of a molar tooth for 14 days.
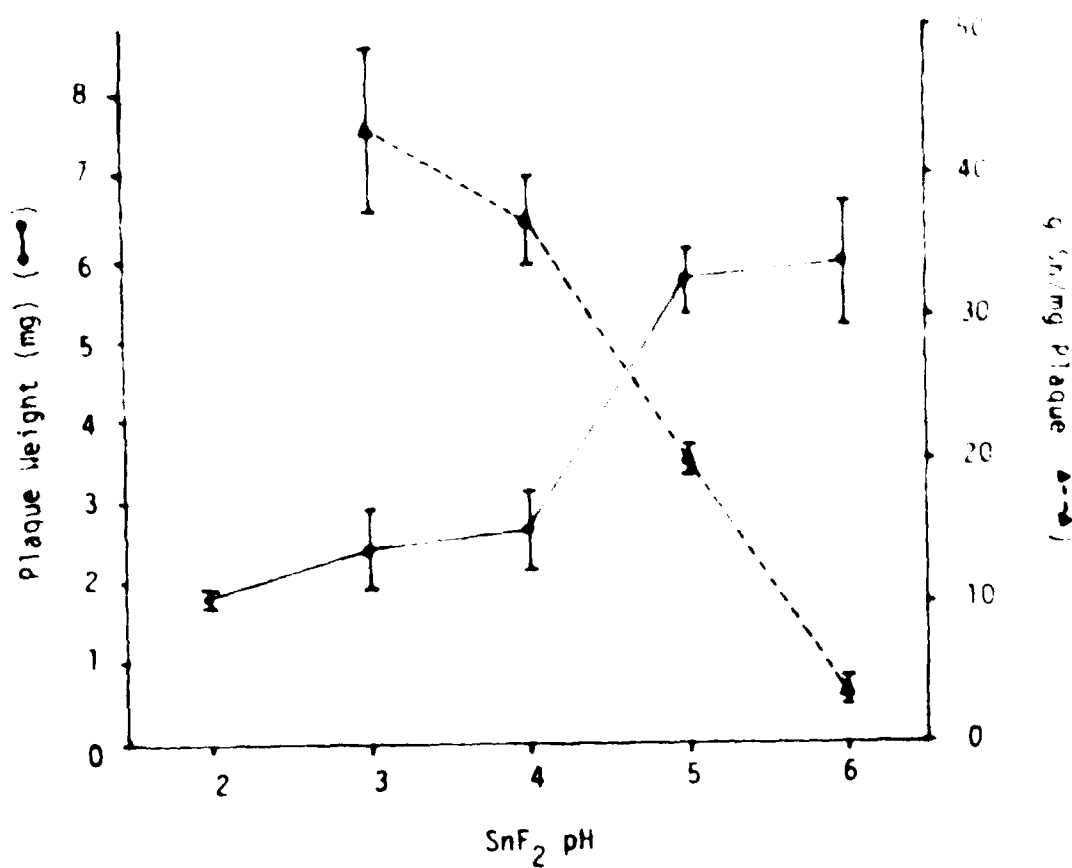
G I

	% Frequency Score 0		% Frequency Score 2	
	Placebo	SnF2	Placebo	SnF2
Baseline	82	88	0	2
Day 7	80	82	0	2
Day 14	46	44	6	9
Post-Trial	81	87	0	1

Table 22   Mean frequency per subject of GI scores 0 and 2 in subjects having slow release SnF2 restoration (N=7) or a placebo restoration (N=7) of a molar tooth for 14 days.

185

Figure 1: Increasing the pH of SnF$_2$ decreases its effectiveness as shown by the greater plaque accumulation on wires and the reduced tin per mg plaque.
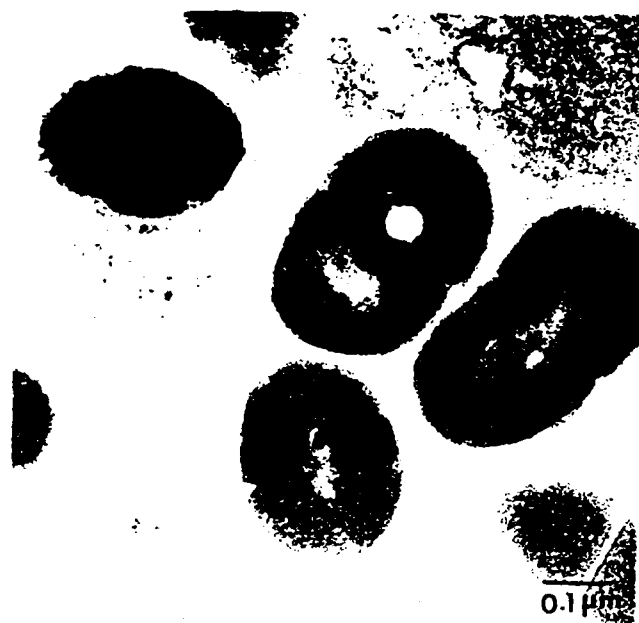
Figure 2: Electron micrograph of S. mutans exposed to NaF (250 ppm F⁻).
Unstained, X80,000

AD-A183 698   FIELD APPLICABLE METHOD TO REDUCE DENTAL EMERGENCIES   3/3
             (U) CONNECTICUT UNIV HEALTH CENTER FARMINGTON
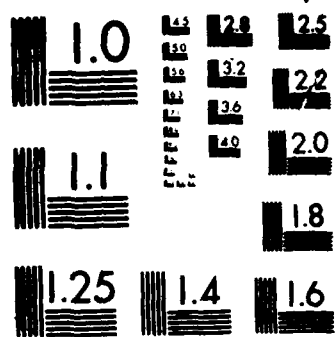   N TINANOFF ET AL. 31 JUL 87 DAMD17-81-C-1075

UNCLASSIFIED   F/G 6/5   NL

END
9-87
DTIC

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963-A

Figure 3: Electron micrograph of S. mutans exposed to SnF₂ (250 ppm F⁻). Note frequent intracellular electron dense granules (black arrows) and electron lucent holes (white arrows). Unstained, X80,000
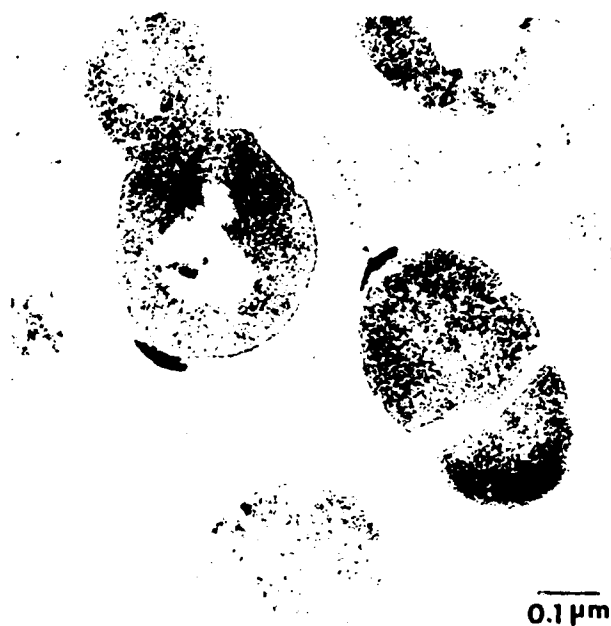
Figure 4: Electron micrograph of <u>S. mutans</u> exposed to PbF$_2$ (100 ppm F$^-$).
Note electron dense granules outside the bacteria.
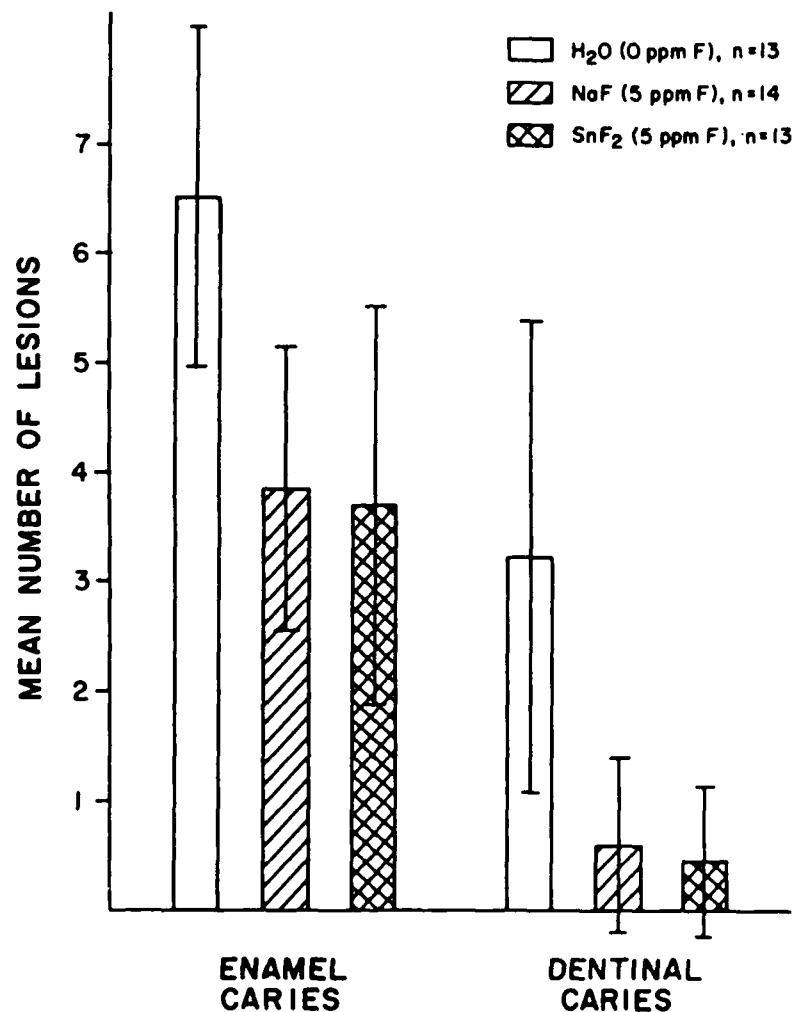Unstained, X80,000

Figure 5: Means and standard deviations of enamel and dentinal carious lesions per hamster drinking either deionized H_2O, NaF, or SnF_2 (5 ppm F-). Experimental caries was produced by oral inoculation of streptomycin-resistant S. mutans NCTC 10449 and by NIH diet 2000. Exposure to fluoride started when animals were approximately 38 days old to provide essentially a topical effect.
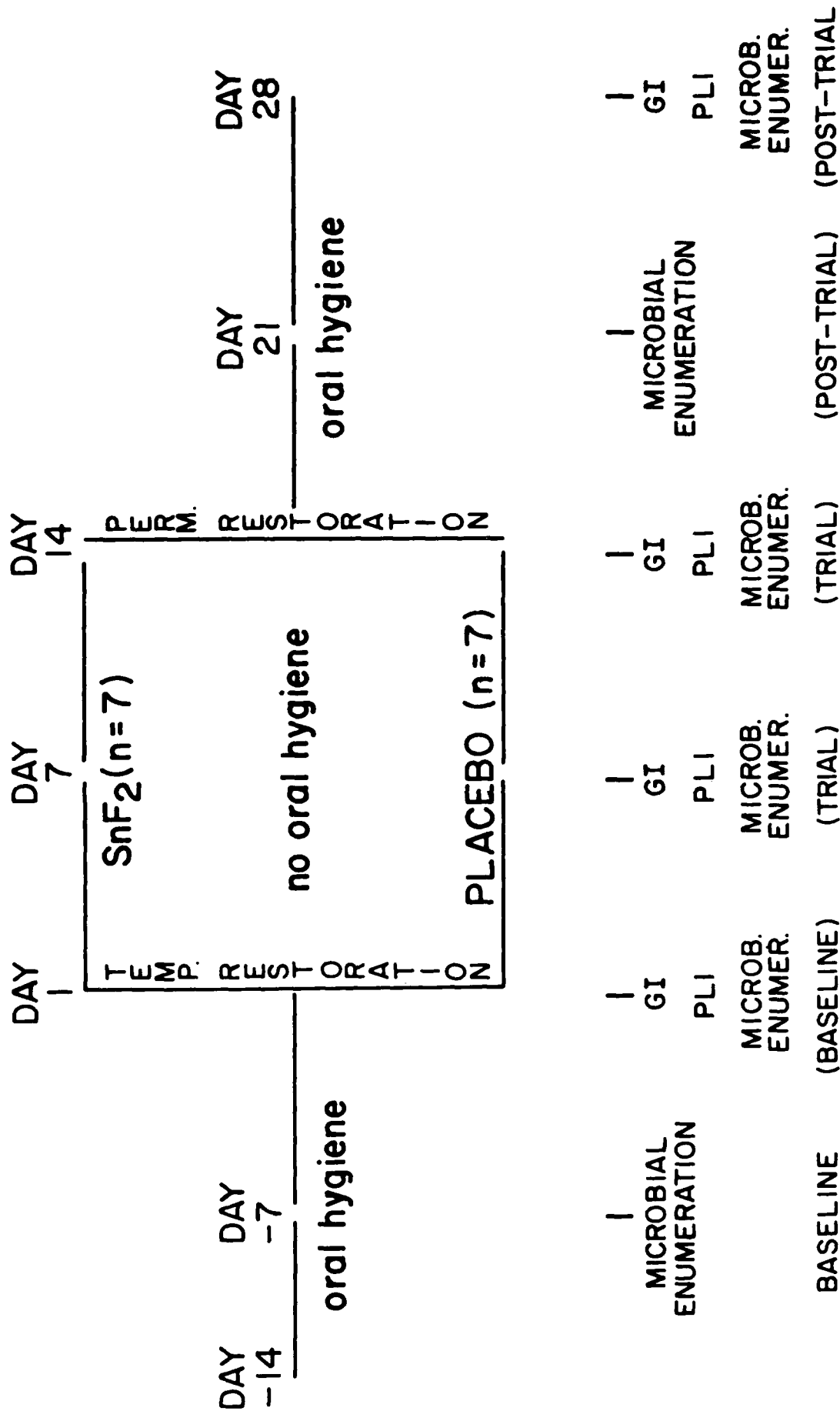
DAY 1

DAY 7

DAY 14

DAY 21

DAY 28

SnF₂ (n=7)

TEMP. RESTORATION

no oral hygiene

PLACEBO (n=7)

PERM. RESTORATION

oral hygiene

DAY -14

DAY -7

oral hygiene

—
GI
PLI

MICROBIAL ENUMERATION

—
GI
PLI
MICROB. ENUMER.

—
GI
PLI
MICROB. ENUMER.

—
GI
PLI
MICROB. ENUMER.

—
GI
PLI

MICROBIAL ENUMERATION

—
GI
PLI
MICROB. ENUMER.

MICROBIAL ENUMERATION

BASELINE

BASELINE)

(TRIAL)

(TRIAL)

(POST-TRIAL)

(POST-TRIAL)

Figure 6: Experimental design for efficacy trial. After an initial preparation period of 14 days, subjects suspended oral hygiene and received either a SnF2 polycarboxylate restoration or a placebo restoration in 1 molar tooth. Following 14 more days, oral hygiene was reinstituted and permanent restorations were placed. Data collection was taken twice during the initial preparation, trial, and post-trial periods.
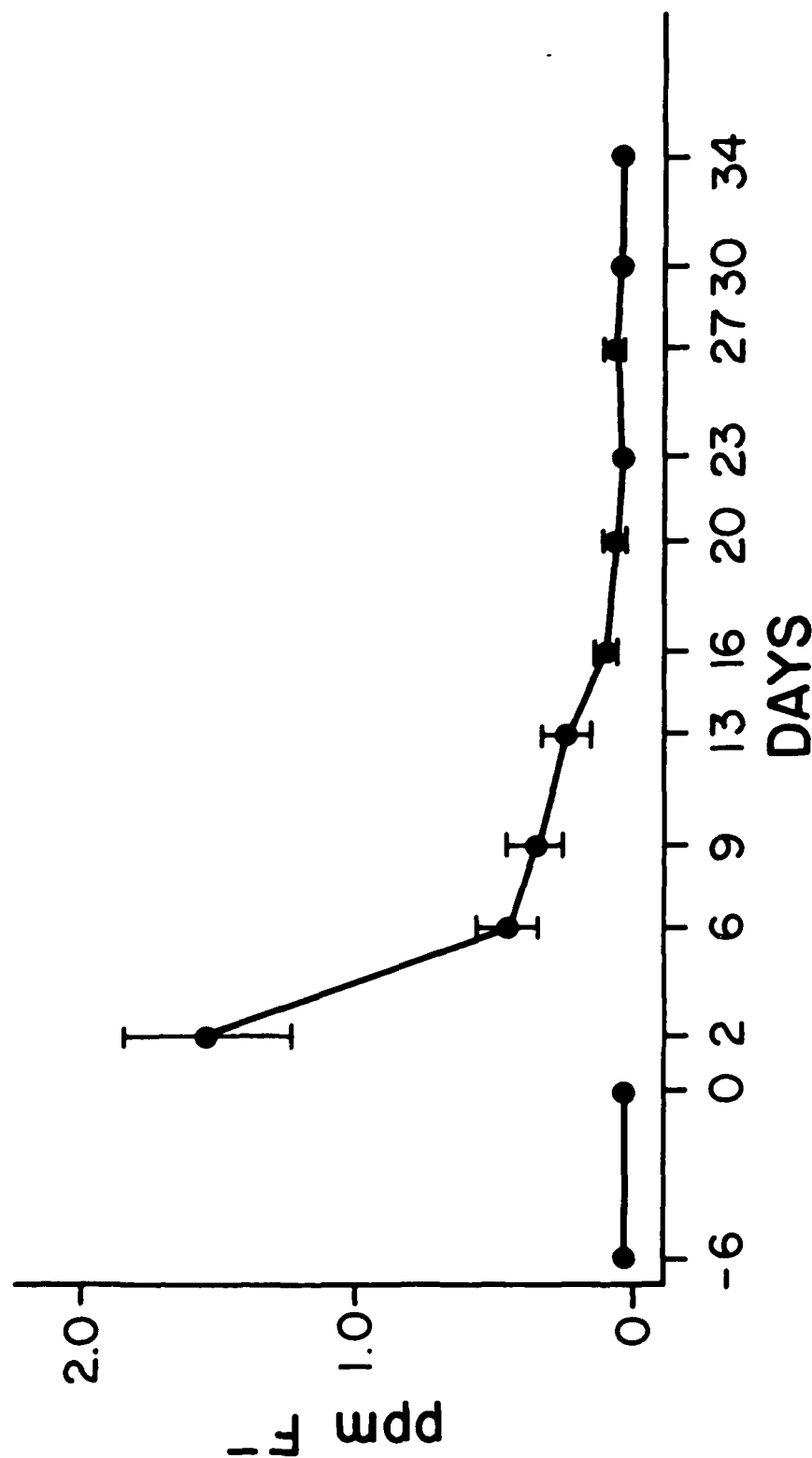
191

Figure 7: Salivary fluoride levels (mean and S.D.) from 7 subjects in the trial for safety. SnF_2-polycarboxylate cement was placed in a molar tooth on day 0 and removed on day 34. Trial was preceded by 2 baseline levels.
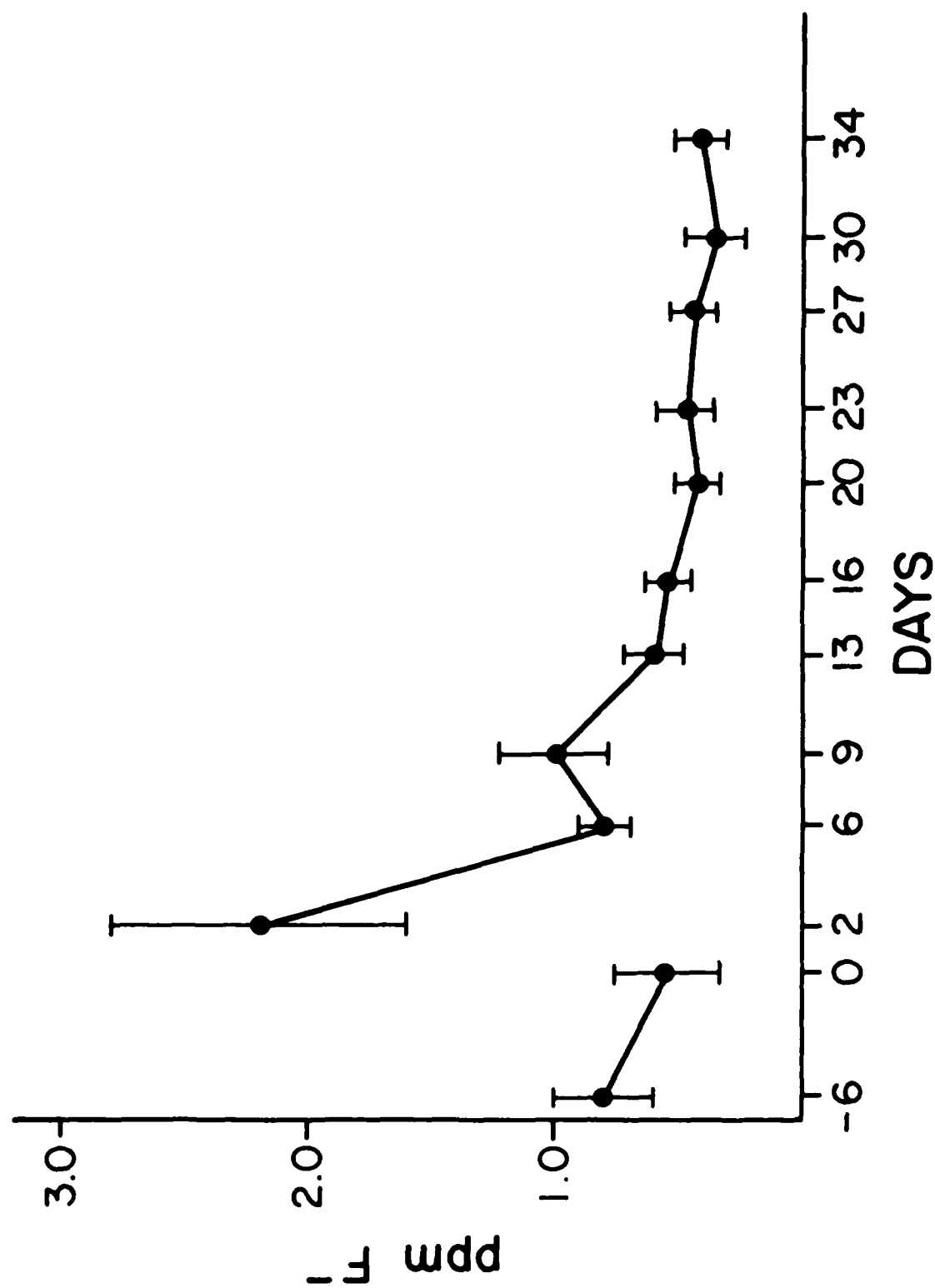
Figure 8: Urinary fluoride levels (mean and S.D.) from 7 subjects in the trial for safety. SnF2-polycarboxylate cement was placed in a molar tooth on day 0 and removed on day 34. Trial was preceded by 2 baseline levels.

Figure 9: Condition of the SnF_2-polycarboxylate restoration after 34 days in a "MOD" cavity preparation in a first permanent molar.

END
9-87
DTIC